A.M.A.

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INDEX NUMBER
IN TWO SECTIONS — SECTION I

JUNE 1956

VOLUME 61 NUMBER 6

auma and Cancer
R. H. Rigdon

ngbinding of Skeletal Muscle
Robert E. Perry Jr., Albert G. Smith, and Richard N. Wrenn

udies on Experimental Hypertension and Cardiovascular Disease
J. L. Orbison, Evelyn Peters, and C. L. Christian

herosclerosis
Edwin F. Hirsch and Richard Nailor

ne Mechanism of Shock Produced by Means of Staphylococcal Toxin
Alan P. Thal and W. Egner

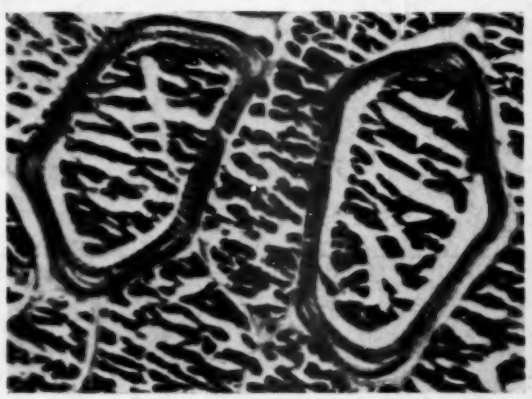
emosiderin Deposition in Early Atherosclerotic Plaques
J. C. Paterson, T. Moffatt, and Jean Mills

omeostasis of Connective Tissues
Hubert R. Catchpole, Norman R. Joseph, and Milton B. Engel

ardiac Cirrhosis
Harry B. Neustein

Production of Aortic Rupture in Rats Fed Purified Diets and beta-Aminopropionitrile
Joseph J. Lalich

Complete Contents on First Inside Page



From Perry et al., p. 450

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TABLE OF CONTENTS

VOLUME 61 JUNE 1956 NUMBER 6

ORIGINAL ARTICLES

PAGE

Trauma and Cancer

R. H. Rigdon, M.D., Galveston, Texas 443

Ringbinding of Skeletal Muscle

Robert E. Perry Jr., M.D.; Albert G. Smith, M.D., and Richard N. Wrenn, M.D., Durham, N. C. 450

Studies on Experimental Hypertension and Cardiovascular Disease

J. L. Orbison, M.D., Rochester, N. Y.; Evelyn Peters, M.D., Kansas City, Kan., and C. L. Christian, M.D., Cleveland, with the Technical Assistance of Kate Gruen 456

Atherosclerosis

Edwin F. Hirsch, M.D., and Richard Nailor, Ph.D., Chicago 469

The Mechanism of Shock Produced by Means of Staphylococcal Toxin

Alan P. Thal, M.B., Ch.B., and W. Egner, B.S., Minneapolis 488

Hemosiderin Deposition in Early Atherosclerotic Plaques

J. C. Paterson, M.D.; T. Moffatt, and Jean Mills, M.A., London, Ont., Canada 496

Homeostasis of Connective Tissues

Hubert R. Catchpole, Ph.D.; Norman R. Joseph, Ph.D., and Milton B. Engel, D.D.S., Chicago 503

Cardiac Cirrhosis

Harry B. Neustein, M.D., Denver 512

Production of Aortic Rupture in Rats Fed Purified Diets and beta-Aminopropionitrile

Joseph J. Lalich, M.D., Madison, Wis. 520

REGULAR DEPARTMENTS

News and Comment 525

Books 526

A. M. A. ARCHIVES OF PATHOLOGY

VOLUME 61 JUNE 1956 NUMBER 6

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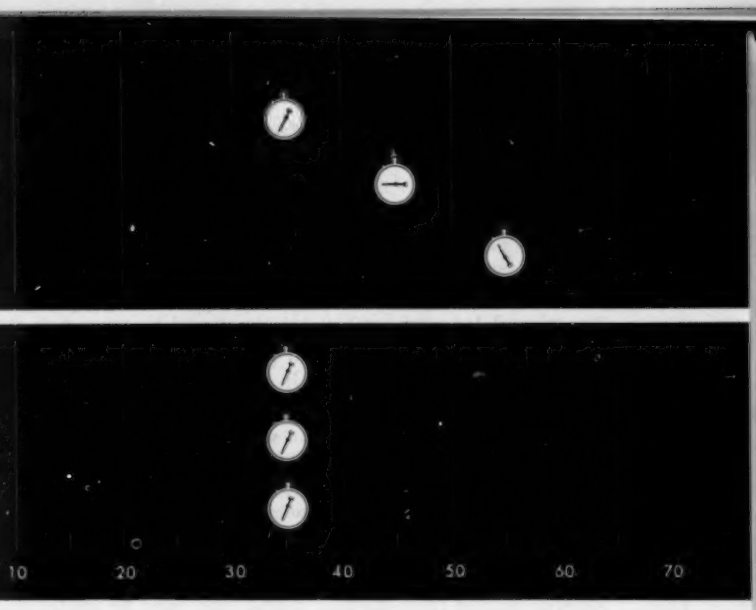

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A.M.A. Archives OF PATHOLOGY

Trauma and Cancer

An Experimental Study in the White Pekin Duck

R. H. RIGDON, M.D., Galveston, Texas

The relation of trauma to cancer has been discussed frequently; however, it is difficult to establish a cause-and-effect relationship. The role of trauma as a co-carcinogen is suggested by both experimental and clinical studies.* The relation between mechanical trauma and carcinogen-induced-tumors has been studied.† Deelman [7] observed that wounds of a tarred area localized and hastened tumor formation. Orr [10] also found that subcutaneous injuries resulting from linen sutures hastened the development of both tar and dibenzanthracene-induced-tumors. The experimental studies of Rous and associates [8] lend support to the opinion that injury of a carcinogen-prepared area may precipitate tumor appearance.

In previous studies it has been shown that a variety of neoplasms develop in the skin of white Pekin ducks following local applications of methylcholanthrene.‡ During the time of these studies we were impressed by the fact that hemangiomas frequently occurred in those areas of skin where large feathers had been plucked. An experiment was designed to study the relation of trauma, as manifested by the plucking of feathers, to the development of tumors in the skin of white Pekin ducks treated with methylcholanthrene. The results of these observations are now reported.

Submitted for publication Feb. 15, 1956.

Laboratory of Experimental Pathology, University of Texas Medical Branch, Galveston, Texas.

This investigation was supported in part by a research grant C-1469 (C3) from the National Cancer Institute, of the National Institutes of Health, Public Health Service, and the Tobacco Industry Research Committee.

* References 1 through 6.

† References 7 through 10.

‡ References 11 through 14.

METHODS AND MATERIAL

White Pekin ducks varying in age from 12 to 45 days were used. They were obtained when one day of age from a commercial hatchery. The young birds were kept in an indoor room, and later they were put in a large outside pen with access to a pool of water. The ration consisted of duck Startena and Growena (Purina) supplemented with cracked corn.

The carcinogen was a 0.25% solution of methylcholanthrene in acetone. It was applied to the skin of the body beneath the right wing and the adjacent inner surface of the same wing. The amount of methylcholanthrene applied daily to the skin of the different birds and the number of applications are given in the specific experiments. The feathers were plucked once a week from the methylcholanthrene-treated area of skin of specific groups of birds. The age of the ducks and the time the feathers were plucked are given in the specific experiments. Acetone was applied to the skin of 10 ducks in a manner similar to that of the methylcholanthrene and covered a comparable area. The feathers were plucked from 10 normal birds for a second control experiment.

The ducks were observed usually once a week for the presence of tumors. Papillomas and hemangiomas were recognized easily; however, the solid tumors could not be correctly diagnosed as fibromas or neurogenic tumors except microscopically, as discussed in a previous paper.[14] Therefore, all solid tumors in this particular study are referred to as fibromas. All tumors occurred in the areas of skin treated with that carcinogen, except for some that

TABLE 1.—*Groups of Ducks Treated with Methylcholanthrene for Varying Periods of Time with Feathers Plucked and Unplucked*

Groups	No. of Ducks per Group	No. of Applications of M. C.*	Feathers Plucked	Tumors	No. of Ducks with Tumors	Types of Tumors	Comments
A	5	1	+	0	0	0	Plucked feathers beginning 120 days after methylcholanthrene first applied
B	5	1	0	0	0	0	.....
C	5	2	+	0	0	0	Plucked feathers beginning 120 days after methylcholanthrene first applied
D	5	2	0	0	0	0	.....
E	5	10	+	0	0	0	Plucked feathers beginning 120 days after methylcholanthrene first applied
F	5	10	0	0	0	0	.....
G	4	10	+	+	2	Hemangiomas Fibromas	Plucked feathers beginning 45 days after methylcholanthrene first applied
H	6	10	0	0	0	0	.....
I	3	20	+	+	3	Hemangiomas Fibromas Papillomas	Plucked feathers beginning 45 days after methylcholanthrene first applied
J	7	20	0	+	3	Fibromas Hemangiomas	.....
K	1	30	+	+	1	Hemangiomas Fibromas	Plucked feathers beginning 45 days after methylcholanthrene first applied
L	9	30	0	+	6	Papillomas Fibromas Hemangiomas	.....

* 0.25% solution of methylcholanthrene in acetone.

TABLE 2.—*Frequency and Time of Appearance of Tumors in Ducks Treated with Methylcholanthrene*

Group X: Feathers Plucked During the Time the Carcinogen Was Being Applied and Also Afterward
Group Y: Feathers Were Never Plucked
Group Z: Feathers Were Plucked After the Carcinogen Was Discontinued

		Groups of Ducks		
		X	Y	Z
Number of ducks in each group		29	14	15
Age in days when methylcholanthrene first applied		17	17	14
Feathers plucked		+	0	+*
Number of ducks that developed hemangiomas on body		26	7	14
Total number of hemangiomas developed		92	17	38
Experimental day when hemangiomas first appeared		60	106	84
Number of ducks that developed one or more papillomas on body		25	14	13
Experimental day when papillomas first appeared		36	46	43
Number of ducks that developed fibromas on body		8	3	8
Total number of fibromas developed		13	3	14
Experimental day when fibromas first appeared		36	60	52
Number of ducks that developed one or more hemangiomas on feet		8	5	15
Experimental day when hemangiomas on feet first appeared		96	117	111
Number of ducks that developed one or more papillomas beneath bill		10	6	11
Experimental day when papillomas beneath bill first appeared		104	106	84
Number of hemangiomas that developed on body per bird	1	3	2	3
	2	5	2	5
	3	6	2	4
	4	3	0	1
	5	6	1	0
	6	2	0	0
	7	1	0	0
	8	0	0	0
	9	0	0	1
Experimental day when ducks either died or were killed	42	1	0	0
	84	0	1	0
	132-147	5	0	0
	148-162	6	0	0
	163-179	5	7	1
	180-194	10	2	5
	195-209	0	4	0
	227	0	0	9

* Started after the last application of methylcholanthrene.

developed on the web of the feet and about the head because the birds contaminated these areas while preening. The type of tumor, the total number, and the time (experimental daytime that methylcholanthrene was first applied to the skin) of their first appearance are shown in Table 2.

EXPERIMENTAL FINDINGS

The feathers were plucked from 10 normal ducks, beginning when they were 45 days of age. After 157 days no tumors were present, and the birds were discarded. Ten ducks, 12 days of age, were plucked on the first day that acetone was applied to the skin. The local applications of acetone were continued daily for 30 days. After the last application of acetone, the feathers were again plucked once each week until the 133rd experimental day. At that time there were no tumors, and this group of ducks was discarded.

Thirty ducks, 20 days of age, were used to study the skin response to the varying amounts of the carcinogen. The feathers were plucked only once, immediately preceding the first application of the methylcholanthrene. An amount of 0.5 ml. of a 0.25% solution of methylcholanthrene in acetone was applied one time to the skin of 10 of these birds. A second group of 10 ducks was treated twice, and a third group of 10 birds was similarly treated for 10 consecutive days. At 127 days following the first application of the methylcholanthrene to these 30 ducks, the feathers were plucked from 5 birds in each of the three groups (Table 1). They were plucked once each week during the remaining 90 days. No tumors developed in any of the 30 ducks in Groups A, B, C, D, E, and F (Table 1). In a second experiment 30 ducks, 17 days of age, Groups G, H, I, J, K, and L, were similarly treated with methylcholanthrene. Table 1 also gives the number of plucked and nonplucked birds used in this experiment. The feathers were plucked from the birds in Groups G, I, and K on the 45th experimental day, and thereafter once each week. Six ducks (Group H), as shown in Table 1, were treated 10 times, and no tumors developed. Two of the four ducks (Group G) similarly treated and with the feathers plucked developed local tumors. Ten ducks (Groups I and J) were treated with the same carcinogen for 20 days. The feathers from three of these birds (Group I) were plucked, and each duck developed tumors. Three ducks developed tumors in the group of seven (Group J) that did not have the feathers plucked. Ten ducks were treated for 30 days (Groups K and L). The feathers were plucked from one duck (Group K), and he developed local tumors. Six of the nine birds (Group L) with the feathers intact developed tumors.

From the above experiment, it would seem that 20 applications of methylcholanthrene is about the minimum number of applications necessary to produce tumors on the body, and that plucking of the feathers in methylcholanthrene-treated birds results in the development of a higher percentage of ducks with tumors. To study further this problem of the relation of trauma to the development of tumors, 43 ducks 17 days of age were used (Table 2). The feathers were plucked one time immediately before the methylcholanthrene was first applied. The feathers also were plucked once each week from the 29 ducks in Group X, while the methylcholanthrene was being applied, and also weekly after the local applications of methylcholanthrene were discontinued until the end of the experiment. The feathers were not plucked from the 14 ducks in Group Y. A third group of 15 ducks (Group Z), 14 days of age, was similarly treated with methylcholanthrene, and the feathers were plucked once per week, beginning on the day following the last application of methylcholanthrene. The time of occurrence and the total number of hemangiomas (Figs. 1 and 2), fibromas (Figs. 1 and 3), and papillomas (Figs. 4 and 5) in the three groups of ducks (Groups X, Y, and Z) are shown in Table 2. The frequency of tumors on the web of the feet and beneath the bill of these ducks also is shown in this Table. The percentage of ducks developing hemangiomas on the body is approximately the same in the two Groups X and Z, where the feathers were plucked. Only one-

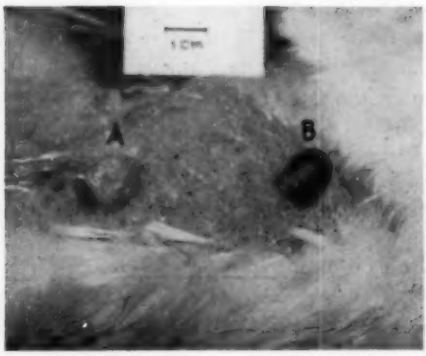


Fig. 1 (Duck 462).—A solid tumor is shown at *A*. Such lesions were considered macroscopically as fibromas in this study. Similar lesions microscopically were found to be lipomas, fibromas, or neurogenic tumors. A typical hemangioma is shown at *B*.

half of the ducks developed tumors where the feathers were not plucked. Furthermore, the hemangiomas on the body appeared much earlier (60 days) in the ducks where the feathers were plucked at the same time that the methylcholanthrene was being applied,

Fig. 2 (Duck 145).—The pattern of growth of the hemangiomas varied widely in these experimental lesions. This illustrates one of the types. Hematoxylin and eosin; reduced about ¼ from mag. × 90.

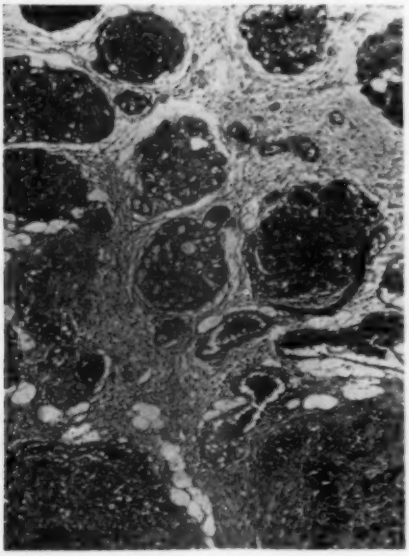
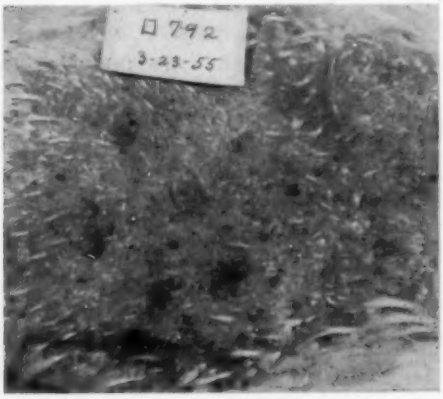


Fig. 3 (Duck 265).—This represents a typical fibroma. Note the many collections of lymphocytes throughout this lesion. Hematoxylin and eosin; reduced about ¼ from mag. × 48.

and in 106 days, where the feathers were not plucked. The fibromas were more frequent in the ducks when the feathers were plucked than they were in the nonplucked birds. They also occurred earlier in the plucked duck. The number of hemangiomas per bird that developed on the feet and the number of papillomas per bird that developed beneath the bill of the plucked (Groups X and Z) and the nonplucked birds (Group Y) were approximately the same. A higher percentage of the birds in Group Z showed papillomas beneath the bill and hemangiomas on their feet than that shown in Group X. This increase may

Fig. 4 (Duck 792).—In many ducks the papillomas were too numerous to count. It is difficult here to establish the relation of the papilloma to the feather follicle.



have been influenced by the fact that this group of 15 ducks (Group Z) was observed for a longer time (Table 2). The time was approximately the same when papillomas first appeared beneath the bill and hemangiomas first appeared on the web of the feet of the ducks in Group X, when the feathers were plucked, as in Group Y, when the feathers were not plucked.

Hemangiomas continued to appear in the skin of the body of the ducks for a much longer time when the feathers were plucked. The number of tumors per bird also was greater in the plucked groups (X and Z)

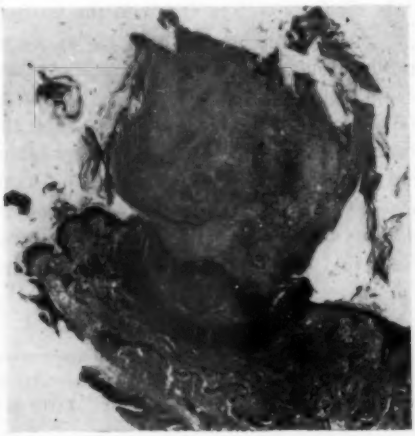


Fig. 5 (Duck 480).—This illustrates the typical papillomas occurring in the duck following the local application of methylcholanthrene. Hematoxylin and eosin; reduced about ¼ from mag. × 18.

(Table 2). The birds in Group X, with feathers plucked, were killed earlier than those in Group Y, in which the feathers were not plucked. Therefore, the above variations may not be attributed to the length of the experiment.

Papillomas developed in the methylcholanthrene-treated areas of skin in the majority of the ducks regardless of whether the feathers were plucked or not. No attempt was made to count the number of papillomas, since they were too numerous in many of the birds (Fig. 4). The time of the first appearance of the papilloma on the body was approximately the same in the three groups X, Y, and Z (Table 2).

COMMENT

In this experiment it has been found that papillomas, hemangiomas, and fibromas occur in and on the skin of the body of white Pekin ducks after the local applications of a 0.25% solution of methylcholanthrene in acetone. The frequency of development of these tumors is related to the number of applications of a specific concentration of this carcinogen applied to the skin. Twenty daily applications of a 0.25% solution of methylcholanthrene in acetone to the skin of the body appear to be approximately the minimum number for the production of these tumors, as suggested by the fact that three ducks from a group of seven thus treated developed fibromas and hemangiomas. When the same concentration of methylcholanthrene was applied to the skin only 10 times and 45 days after the first application of the carcinogen the feathers were plucked, tumors developed in two of the four ducks (Table 1). Thus it appears that trauma, as manifested by the plucking of feathers, influences the development of hemangiomas in the skin of the duck treated with this specific carcinogen. Of the ducks in Group Y, 50% developed hemangiomas on the body, although the feathers were not plucked, whereas 90% of the birds in Group X and 93% of the birds in Group Z with feathers plucked developed hemangiomas.

It is of interest to observe that the number of ducks developing hemangiomas was the same, whether the feathers were plucked at the same time the carcinogen was applied, or if they were plucked following the last application of methylcholanthrene. The time of the first appearance of the hemangiomas was much earlier (60 days) when the feathers were plucked during the time the methylcholanthrene was being applied than it was when the feathers were not plucked (106 days). This observation would suggest that the trauma was a significant factor in the development of these hemangiomas. Fur-

thermore, tumors occurring after the last application of the carcinogen would indicate the effect of trauma to be that of a co-carcinogen. This observation also would suggest that the quantity of methylcholanthrene contacting the skin of the duck may not be as significant in the development of the hemangiomas as the co-carcinogenic effects produced by subsequent trauma. The amount of methylcholanthrene in acetone reaching the skin, no doubt, was less when applied to ducks with their feathers not plucked, as in Group Z, than to ducks where the feathers were plucked both during the time of application of the methylcholanthrene and afterward, as shown by the birds in Group X (Table 2). The observation that there are hemangiomas in ducks treated with methylcholanthrene with their feathers plucked and in ducks similarly treated without the feathers being plucked is of interest, in view of the observations of others referable to tumor formation in the skin of mice.§ Lacassagne and Latarjet [20] have pointed out that "a skin zone (in mice) free from hair follicles and sebaceous glands appeared refractory to the carcinogenic action of methylcholanthrene." In this study on the duck it has been shown that papillomas and hemangiomas develop where there were no feather follicles and sebaceous glands, that is, on the web of the foot and that portion of the undersurface of the bill devoid of feathers. Liang and Cowdry [19] recently discussed the significance of the hair follicles in the pathogenesis of papillomas and carcinomas in the mouse. Simpson and Cramer [16] also have emphasized the fact that the hair follicle in mice is a portal of entry for methylcholanthrene in benzene. In the duck, feather follicles do appear to contribute to the pathogenesis of tumors; however, they are not essential for the development of papillomas and hemangiomas.

The spontaneous regression of tumors in the duck presents some difficulty in a study such as this; however, many of the tumors did persist, becoming 2 to 3 cm. in diameter. The mechanism of regression of hemangiomas and carcinomas in the chicken and duck has been reported.‖ The pathogenesis of these hemangiomas is now being studied, and it would seem that methylcholanthrene stimulates the production of angioblasts, probably through its effect on histiocytes.

§ References 15 through 20.

‖ References 11 and 13.

SUMMARY

Hemangiomas, papillomas, and fibromas have occurred in the skin of the body wall of the duck after local applications of a 0.25% solution of methylcholanthrene in acetone. The number of such tumors is greater in ducks with their feathers plucked than in birds similarly treated and with the feathers not plucked. Although tumors may occur without trauma, the plucking of feathers does play a significant role as a co-carcinogen, as shown in these experiments. Although the feather follicles in the ducks are important in the pathogenesis of tumors, papillomas and hemangiomas do develop on the web of the foot and beneath the lower bill in areas that are devoid of feather follicles and sebaceous glands.

REFERENCES

1. Berenblum, I.: Mechanism of Carcinogenesis: Study of the Significance of Carcinogenic Action and Related Phenomena, Cancer Res. **1**:807-814, 1941.

2. Berenblum, I., and Shubik, P.: New, Quantitative, Approach to the Study of the Stages of Chemical Carcinogenesis in the Mouse's Skin, Brit. J. Cancer **1**:383-391, 1947.

3. Friedewald, W. F., and Rous, P.: Initiating and Promoting Elements in Tumor Production: Analysis of the Effects of Tar, Benzpyrene, and Methylcholanthrene on Rabbit Skin, J. Exper. Med. **80**:101-126, 1944.

4. Rous, P., and Kidd, J. G.: Conditional Neoplasms and Subthreshold Neoplastic States: Study of the Tar Tumors of Rabbits, J. Exper. Med. **73**:365-390, 1941.

5. Kotin, P., and Kahler, J. E.: Possible Role of Trauma as a Cocarcinogen: Case Report on an Oil Worker, Cancer **6**:266-268, 1953.

6. Hueper, W. C.: Medicolegal Aspects of Cancer, Am. J. Clin. Path. **25**:116-125, 1955.

7. Deelman, H. T.: Tar Cancer and Regeneration of Cells, Ztschr. Krebsforsch. **21**:220, 1924, cited by Will, R. A.: Pathology of Tumors, St. Louis, The C. V. Mosby Company, 1948.

8. Rous, P., and Kidd, J. A.: Comparison of Virus-Induced Rabbit Tumors and the Tumors of Unknown Cause Elicited by Tarring, J. Exper. Med. **69**:399-424, 1939.

9. Shear, M. J.: Studies in Carcinogenesis, Am. J. Cancer **33**:499-537, 1938.

10. Orr, J. W.: Effect of Interference with the Vascular Supply of the Induction of Dibenzanthracene Tumors, Brit. J. Exper. Path. **16**:121-126, 1935.

11. Rigdon, R. H.: Spontaneous Regression of Neoplasms: Experimental Study in the Duck, South. M. J. **47**:303-310, 1954.

12. Rigdon, R. H.: Fibroma Arising from Feather Follicle of Duck Following Local Application of Methylcholanthrene to Skin, Proc. Soc. Exper. Biol. & Med. **83**:34-36, 1953.

13. Rigdon, R. H.: Spontaneous Regression of Hemangiomas: Experimental Study in the Duck and Chicken, Cancer Res. **15**:77-79, 1955.

14. Rigdon, R. H.: Neurogenic Tumors Produced by Methylcholanthrene in the White Pekin Duck, Cancer **8**:906-915, 1955.

15. Lauridsen, J., and Eggers, H. E.: Carcinogenesis After Multiple Irritation, Cancer Res. **3**:43-46, 1943.

16. Simpson, W. L., and Cramer, W.: Fluorescence Studies on Carcinogens in the Skin: I. Histological Localization of 20-Methylcholanthrene in Mouse Skin After a Single Application, Cancer Res. **3**:362-369, 1943.

17. Simpson, W. L., and Cramer, W.: Fluorescence Studies of Carcinogens in Skin: II. Mouse Skin After Single and Multiple Applications of 20-Methylcholanthrene, Cancer Res. **5**:449-463, 1945.

18. Glucksmann, A.: Histogenesis of Benzpyrene-Induced Epidermal Tumors in the Mouse, Cancer Res. **5**:385-400, 1945.

19. Liang, H., and Cowdry, E. V.: Role of Hair Follicles in Methylcholanthrene Carcinogenesis in Mice, J. Nat. Cancer Inst. **16**:205-224, 1955.

20. Lacassagne, A., and Latarjet, R.: Action of Methylcholanthrene on Certain Scars of the Skin of Mice, Cancer Res. **6**:183-188, 1946.

Ringbinding of Skeletal Muscle

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Our attention was initially focused on ringbinding of skeletal muscle when we observed curious rings of striated muscle binding the main elongate fascicle of muscle in a patient suffering with spastic muscles. Ringbinding was first described by Heidenhain in the German literature in 1918.[1] However, an extensive search of the English literature failed to reveal information on this problem. It has been the topic of a few German reports since 1918.*

RINGBINDING IN MAN

The following four cases, the first of which is reported in detail, have all shown ringbinding of skeletal muscles.

REPORT OF CASES

CASE 1.—A 15-year-old Cuban boy had a normal birth and early childhood. At age 5 the child had difficulty in talking, and there was questionable mental retardation. He was two years behind children of his own age in school. At age 8 he developed progressing flexion of the fourth and fifth fingers of his right hand. One year later the same flexion of the fourth and fifth fingers appeared in the left hand. When the child was first seen by us at age 15, there was severe flexion contractures of these fingers, bilaterally, severer on the right. There was classical bilateral spastic pes planus with tight Achilles tendons and rigid feet. He was a muscular, active person, and muscle power was present in his lower extremities. There was moderate calf atrophy, bilaterally. A fusion of the proximal interphalangeal joints of the fourth and fifth fingers, and a triple arthrodesis of the right foot was done. A biopsy of the grossly normal peroneal muscle on the right showed many ringbindings (Figs. 1 and 2). The wound healed well after surgery. The patient was seen one year later, and he had excellent functional results and no further progression of the disease.

CASE 2 (S-2405).—A Negro man, age 41, had paresthesias of both lower extremities of some four to six months' duration, with no other complaints referable to the central nervous system. The final diagnosis was peripheral neuropathy, cause undetermined. A right gastrocnemius muscle biopsy was normal except for the presence of ringbinding.

CASE 3 (S-1841).—A white man, age 30, had a compound fracture of the left femur in 1949, with a chronic osteomyelitis following this. There was a resultant ankylosis of the knee joint. A left quadricepsplasty was done in May, 1954, and tissue from this muscle contained ringbinding.

CASE 4 (S-2313-55).—A white man, age 35, had a below-the-knee amputation for arteriosclerotic vascular insufficiency. The stump became infected and an above-the-knee reamputation was done. The muscles in the amputated stump had ringbinding.

After our attention had been called to ringbinding by the biopsy specimen in Case 1, all of the muscle biopsies in the surgical material of our service since 1953 were reexamined. There were 58 muscle biopsy cases, and our Cases 2, 3, and 4 were found in a study of this material.

The ringbinding seen in our first case was typical. The width of the circular band varied from 5μ to 12μ, with an average of 8μ. The circumferential bands extended for an average of 100μ along the central muscle core (Fig. 3). These bands stained as muscle with the Masson muscle stain, which was used with a routine hematoxylin and eosin in the study of all material. It was shown definitely that the bands had cross striations and were within the sarcolemma of the muscle fascicle. Cresyl violet and Congo red stains for amyloid were negative. Care was taken to distinguish true ringbinding from

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From the Department of Pathology and the Department of Surgery, Duke University School of Medicine, and from the Laboratory Service, Veterans Administration Hospital, Durham, N. C.

* References 2 through 7.

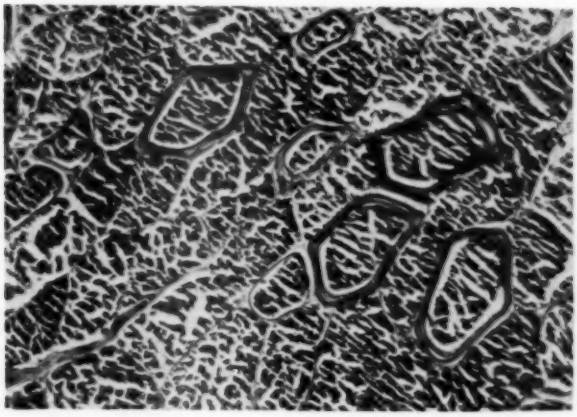

Fig. 1.—Section of muscle in Case 1. Note number of ringbindings; × 226.

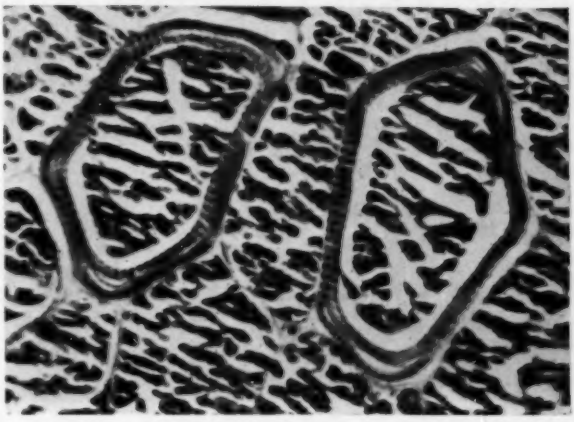

Fig. 2.—Higher magnification of ringbindings seen in Figure 1. The bands clearly show cross striations; × 610.

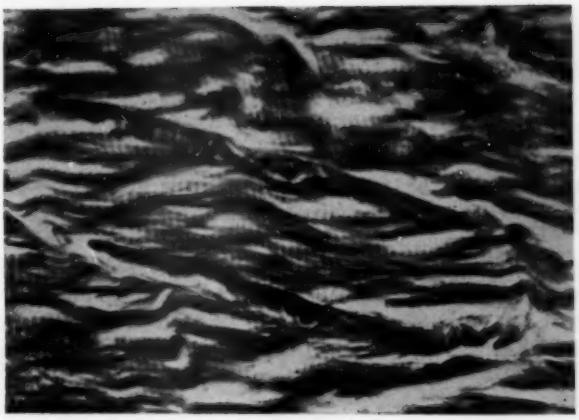

Fig. 3.—Longitudinal cut fascicle with outer binding fibers cut in cross section; × 610.

simple separation of muscle fibers in the fascicle. In the latter instance, the cross striations ran in the same direction as those in the main body of muscle. In addition, some of the circular bands were incomplete, encompassing only a fraction of the diameter of the muscle fascicle (Fig. 4).

Complicated forms of ringbinding were described by Bergstrand [6] as bands running through the muscle fascicle as well as about it. We have seen some of these complicated forms in our material but not nearly as many as simple ring forms.

When found, ringbinding has usually been associated with some abnormality of muscle or disease involving tissues adjacent to the ringbound muscles. Günther reported simple and complicated ring forms in three cases.[7] In the first case, the rings were located in muscles of the leg, which was amputated for arteriosclerotic vascular insufficiency. In the second case, the structures were found in the left diaphragm, over which the pleura was thickened due to an old pleurisy. The third case was a 62-year-old man who had carcinoma of the stomach, and the diaphragm had ring forms present in it. Examination of many biopsies of human muscles by Günther failed to reveal any ringbinding except in the flexor carpi ulnaris muscle of one patient. He does not describe any disease in this patient. Bergstrand described ringbinding of the complicated and simple type in the muscles of more than 50% of the cases of myotonia dystrophies studied by him.[6] In the first cases described by Heidenhain, all had some type of muscular dystrophy.†

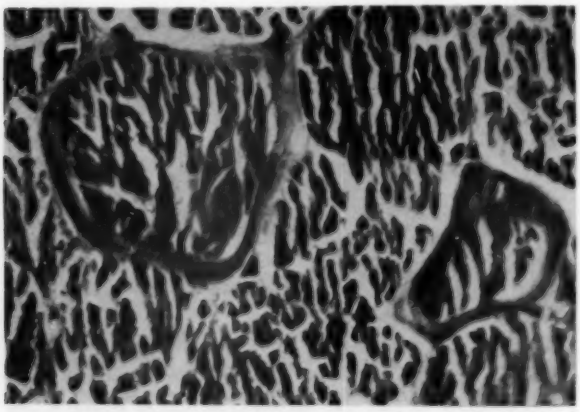

Fig. 4.—Cross section of muscle fascicle with incomplete ringbinding. The fascicle on the left is not completely encircled by the band; × 610.

RINGBINDING IN ANIMALS

To our knowledge, the only report of finding ringbinding in animals was by Günther, who studied ringbinding by examining various muscles of many different animals.[7] He found a single simple form in the diaphragm of a hedgehog and the gastrocnemius of a white rat.

We attempted to produce ringbindings by animal experimentation. Since we believed that this structure represented a change due to spastic contraction of muscles, we attempted to produce ringbinding by the administration of strychnine to experimental animals. For this experiment we used five white mice and five adult guinea pigs. Three of each group of animals were given strychnine, and the other two of each group were used as controls. Biopsy specimens of the diaphragm, rectus muscles of the abdomen, and muscles of the thigh were examined in every instance. The mice were killed by the administration of 1 mg. of strychnine subcutaneously. The guinea pigs were killed by the intracardiac administration of 6 mg. of strychnine. The controls were killed by chloroforming. Death occurred in less than one minute, and the

† References 1 and 2.

muscles were removed immediately. Part of each muscle was fixed immediately in Zenker's formol and Bouin's fluids. The remainder of the muscles were kept moist with isotonic saline and fixed in the above fluids 30 minutes later. The reason for this was to eliminate any artifact that might be produced by the immediate fixation of the muscles. The blocks were embedded in paraffin and sectioned at 6μ. They were stained with hematoxylin and eosin. Rare simple ring forms were found in the rectus muscle of a control guinea pig after immediate fixation in Bouin's fluid, in the diaphragm of a control guinea pig after immediate fixation in Zenker's, and in the rectus muscle of a control guinea pig after immediate fixation in Zenker's (Fig. 5). In no instance were ring forms demonstrated in the muscles of the test guinea pigs or the test and control white mice. There was no instance in the positive material in which these forms were complicated or numerous.

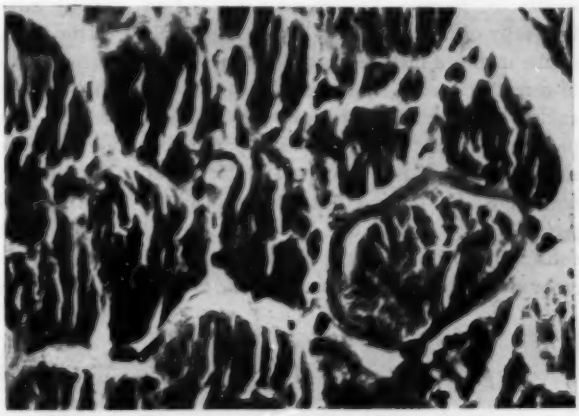

Fig. 5.—Single, simple ringbinding seen in the diaphragm of an experimental animal; × 610.

INTERPRETATION

Heidenhain regarded ringbinding as pathognomonic for muscular dystrophies.‡ Bergstrand, Wohlfart, Günther, and other German investigators have disagreed.§ They have been of the opinion that these forms are more frequent in muscular dystrophies, but that they may be seen in other conditions. All have felt that ringbinding is a pathologic state, however. Adams, Denny-Brown, and Pearson state that ringbindings are artifacts which appear in muscles because of the strong liability of myotonic muscles to such contraction artifacts.[8] It is our conclusion that ringbinding, when seen in numbers, represents a pathological alteration of skeletal muscle due to contracture or hypertonicity of the affected muscle. Ringbindings are seen more frequently in myotonic and other forms of irritable muscle caused by vascular insufficiency, chronic inflammation, or peripheral neuropathies. Ringbinding does not represent any pathognomonic change for any specific disease of muscles, but a pathological change in muscles due to hypertonicity or increased irritability of the affected muscle. Günther has postulated that a humoral or toxic agent may be responsible for this change.[7]

Adams, Denny-Brown, and Pearson refer to ringbinding as a homogenous sarcoplasm about the fascicle caused by transverse section of an ordinary contraction band of an entire fiber.[8] We do not entirely agree with this concept, since cross sections of the simply thickened or buckled contraction bands would not show cross striation at a 90° angle to the central muscle fascicle. It is our concept that the pathogenesis of this change is spiraling of the outer muscle fibers of the involved and

‡ References 1 and 2.

§ References 5, 6, and 7.

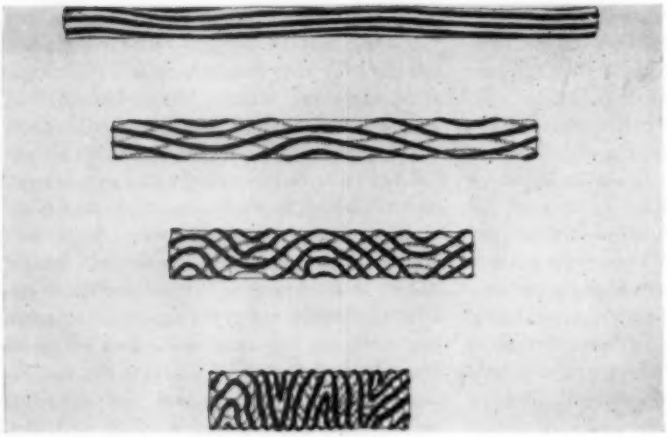

Fig. 6.—Schematic representation of our concept of the formation of ringbinding.

contracted fascicle. This concept is based on the study of the ring forms at different levels of the involved fascicle as well as the actions of a model fascicle. This model is a piece of rubber tubing with strands of wire about the periphery of the tubing running parallel to the long axis of the tubing and attached at both ends. By placing this tubing inside a piece of glass tubing, one has the crude reconstruction of a muscle fascicle. A slight amount of tension on the ends of the rubber tubing causes the outer wires to be parallel to the long axis of the tubing. However, if one compresses the ends of the rubber tubing, as would be the case in a contracted muscle fascicle, the outer wires, or fibers, spiral about the central core (Figs. 6 and 7). The involved fascicle may have to be in a more contracted state over a prolonged period of time to cause the outer fibers to remain spiraled around the fascicle. The formation of the compound type of ringbinding pro-

Fig. 7.—Drawing of muscle fascicle with ringbinding according to our concept of spiraling of the outer fibers. Lower, cross section of fascicle at indicated level.

duced by a spiraling of inner fibers would be explained by the same pathogenesis. We think that the complete band that is seen in some cases is due to a fusion of the spiraled outer fibers.

SUMMARY

Ringbinding of skeletal muscle in humans and experimental animals is reported, apparently for the first time in the English literature.

The presence of ringbinding in abundance occurs when there is hypertonicity or increased irritability of skeletal muscles, especially in primary muscle diseases.

Our concept of the development of ringbinding in striated muscle is that the outer fibers of the fascicle spiral about the central core forming the rings observed in cross sections. This concept is based on the study of sections of ringbinding and of a model fascicle.

Mr. William B. McNett did the drawings for Figures 6 and 7, and also constructed the model used in this study.

REFERENCES

1. Heidenhain, M.: Beobachtungen über die progressiven Veränderungen der Muskulatur bei Dystrophia myotonica, München. med. Wchnschr. **65**:85-86, 1918.

2. Heidenhain, M.: Über progressive Veränderungen der Muskulatur bei Myotonia atrophica, Beitr. path. Anat. **64**:198-225, 1918.

3. Schutz, H.: Über Veränderungen der quergestreiften Muskeln und des retrobulbären Fettgewebes bei Morbus Basedowii, Beitr. path. Anat. **71**: 451-466, 1923.

4. Voss, H.: Das histologische Bild der Axolotlmuskulatur, insbesondere die quergestreiften Ringbinden der Muskelfasern, Ztschr. mikr.-anat. Forsch. **28**:161-184, 1932.

5. Wohlfart, G.: Quergestreifte Ringbinden in normalen Augenmuskeln, Ztschr. mikr.-anat. Forsch. **29**:592-604, 1932.

6. Bergstrand, C. G.: Zur Morphologie der quergestreiften Ringbinden, Ztschr. f. mikr.-anat. Forsch. **44**:45-55, 1938.

7. Günther, P. G.: Einfache und komplizierte Ringbindenformen in der quergestreiften Skelettmuskulatur, Arch. path. Anat. **322**:214-220, 1952.

8. Adams, R. D.; Denny-Brown, D., and Pearson, C. M.: Diseases of Muscle, New York, Paul B. Hoeber, Inc., 1953, p. 277.

Studies on Experimental Hypertension and Cardiovascular Disease

II. The Effects of Fluid and Electrolyte on Bilaterally Nephrectomized Dogs

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In a previous publication we reported the effectiveness of intraperitoneal 0.9% NaCl solution and of balanced salt solution (modified Locke's solution) in the production of both hypertension and arterial necrosis in bilaterally nephrectomized dogs.[1] These phenomena appeared to be induced by changes in fluid and electrolyte balance, but it was noticed particularly that the modified Locke's solution was more effective in inducing arterial disease and hypertension than was 0.9% NaCl. It is the purpose of this paper to report on the fluid and electrolyte changes in those animals previously described and the relative effectiveness of various other electrolyte solutions in producing hypertension and vascular disease.

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METHODS AND MATERIALS

A. Animals

The dogs used in these experiments were obtained from the pound and kept on a standard kennel diet for one to three months. During this period they were trained to the technique of direct femoral artery puncture for blood pressure determination. Daily weights were determined on a Toledo Scale (± 30 gm.)

B. Electrolyte Solutions

The electrolyte solutions used in this study were all autoclaved before injection and had the following compositions:

(1) NaCl 0.9% (150 mEq/L. of both Na and Cl).

(2) A solution described by Skeggs and Leonards [2] and modified by Leonards.* In the previous report this was designated as "electrolyte solution," but it seems more appropriate to use the term "modified Locke's solution." The composition of the solution in ionic milliequivalents is as follows:

Na^+	148 mEq./L.
K^+	3 mEq./L.
Ca^{++}	5 mEq./L.
Mg^{++}	3 mEq./L.
Glucose	100 mg./100 cc.
Cl^-	126 mEq./L.
HCO_3^-	24 mEq./L.
HPO_4^-	2 mEq./L.
Lactate	7 mEq./L.

The solution was made up as two stock solutions, A and B, having the following composition:

Solution A	
KCl	9.1 gm./L.
$CaCl_2$	11.1 gm./L.
$MgCl_2 \cdot 6H_2O$	12.2 gm./L.
NaCl	209.0 gm./L.
Glucose	40.0 gm./L.
Lactic acid	27.1 ml. (85%-90% sol.)

Solution B	
$NaHCO_3$	84.0 gm./L.
Na_2HPO_4	5.7 gm./L.
NaOH	9.6 gm./L.

These solutions were autoclaved and a 25 ml. aliquot of each (A and B) was added to enough sterile purified water to make a final volume of 1 liter.

* Leonards, J. R.: Personal communication.

(3) The mixed cation solution was designed to have the same concentration of cations as the modified Locke's solution, but to have only chloride as the anion. This was prepared as a single stock solution, and 50 cc. was diluted to 1 liter to make the final solution. The composition of the mixed cation solution is as follows:

KCl	4.5 gm./L.
$CaCl_2$	5.5 gm./L.
$MgCl_2$	6.1 gm./L.
NaCl	171.96 gm./L.
Glucose	20.0 gm./L.

(4) The mixed anion solution had the same concentration of each anion as the modified Locke's solution but contained sodium as the only cation. The two stock solutions had the following composition and were diluted as the modified Locke's solution.

Solution A

NaCl	285.4 gm./L.
Glucose	40.0 gm./L.
Lactic acid	27.1 ml./L. (85%-90% sol.)

Solution B

$NaHCO_3$	84.0 gm./L.
Na_2HPO_4	5.7 gm./L.
NaOH	9.6 gm./L.

(5) The low-sodium solution contained the same number of milliequivalents of all the ions as the modified Locke's solution, with the exception of lower values of Na^+ and Cl^- (Na^+=80 mEq/L. and Cl^-=67 mEq/L.). This was effected by reducing the NaCl content of the modified Locke's solution from 269.0 to 112.0 gm/L. Dilution of this stock solution was the same as for the modified Locke's solution.

(6) The hypertonic NaCl solution was made and used in a concentration of 10%.

(7) The hypertonic NaCl·$NaHCO_3$ solution was made so that the Na content was equivalent to the 10% NaCl solution, but half of the Na was in the form of the bicarbonate. Hence, the solution was made up to contain NaCl·50 gm/L. and $NaHCO_3$·72.4 gm/L. The solution was used without dilution. It had been hoped that a "concentrated" modified Locke's solution could be used in comparison with the 10% NaCl. Since it was found that the component salts of the modified Locke's solution were not soluble at such a concentration, this simple concentrated NaCl·$NaHCO_3$ solution was substituted.

C. Procedure

Eight groups of bilaterally nephrectomized dogs were studied. Group 1 consisted of seven dogs given nothing by mouth or intraperitoneally, and the animals of this group were the controls. Group 2 consisted of seven dogs given 0.9% NaCl solution at the rate of 100 ml/kg/day. Group 3a consisted of seven dogs given modified Locke's solution at the rate of 100 ml/kg/day. This procedure was later repeated on five dogs at the time when fluid space studies were being done every second day; these dogs comprised Group 3b. Group 4 consisted of six dogs given the mixed cation solution at the rate of 100 ml/kg/day. Group 5 consisted of six dogs given the mixed anion solution at the rate of 100 ml/kg/day. Group 6 consisted of six dogs given the low-sodium solution at the rate of 100 ml/kg/day. Group 7 consisted of eight dogs given 10% NaCl solution at the rate of 8.0 ml/kg/day. Group 8 consisted of five dogs given the hypertonic NaCl·$NaHCO_3$ solution at the rate of 8.0 ml/kg/day. In both Groups 7 and 8, a solution of 1% procaine was given three to five minutes before the hypertonic solution to prevent pain, usually 10-15 ml.

Plasma sodium and potassium concentrations were determined by use of the Baird flame photometer, and chloride by the method of Whitehorn.[3] Blood volume was determined by the dilution of Evans blue (T-1824) injected intravenously.[4] Inulin was obtained in sterile ampules from U. S. Standard Products Co., of Woodworth, Wis. Each ampule contained 5 gm. in 50 ml. of 0.9% NaCl. An amount of 10 ml. was injected for each determination, and the sample was withdrawn after four hours. The inulin in the plasma was determined by the method of Higashi and Peters.[5]

Blood pressure determinations were made on all animals three times per week until a stable control level was established, usually a period of one to three months. A nephrectomy was then performed and followed one to three weeks later by a second nephrectomy. The time of the second nephrectomy was considered as the beginning of the experimental period. From this time until the end of the experiment blood pressures were determined daily, and in some instances twice daily.

Throughout the experimental period the animals received no food or water by mouth, all intake being by the intraperitoneal route. Since our intent was to overload these animals with electrolyte solutions, all of the animals except the controls were given daily intraperitoneal injections of a predetermined amount of solution. None of the fluid injected was ever withdrawn.

The control determinations for Evans-blue and inulin space were made immediately after the second nephrectomy. In the earlier work only one additional Evans-blue and inulin space determination was made near the end of the experimental period. As the work progressed, it seemed desirable to get more information on these space changes, and therefore determinations were made every second day during the experimental period. Since Evans-blue and inulin continue to circulate in the blood for longer than 48 hours in these animals, the blank determinations were elevated. However, the residual Evans-blue and inulin levels changed very little over the time required for equilibration of the newly

added inulin or Evans blue, and thus we were allowed to make adequate serial determinations.

All animals were autopsied and both macroscopic and microscopic examinations were made of thoracic and abdominal viscera, skeletal muscles, and skin. A rough quantitation of the lesions was made by assigning values from 0 to +++ to the changes in each of four organs: heart, stomach, colon, and urinary bladder. By totaling the "pluses" for the four organs, a number was obtained which was proportional to the degree of tissue damage. In all instances the lesions were similar to those previously reported.[1]

TABLE 1.—*Summary of Changes in Fluid Compartments, Plasma Electrolytes, Blood Pressure, and Vessels in Bilaterally Nephrectomized Dogs* *

	Dogs Given No Fluid (Group 1)						
Dog no.	27	34	35	36	37	39	41
Degree of vascular necrosis	0	0	0	2+	0.5+	0	0.5+
Maximum blood pressure	145	130	150	160	120	120	125
Maximum change in blood pressure	+18	0	+15	+25	0	+ 5	0
Maximum change in Na^+	+ 4	+ 5	0	+ 7	+ 5	0	+ 5
Maximum change in K^+	+ 4.4	+ 5.3	+ 4.8	+ 5.2	+ 4.7	+ 5.6	+ 3.7
Maximum change in Cl^-	−13	−17	0	−15	− 5	−22	− 3
Change in blood volume	+37	−52	..	−375	+79	+15	+22
Change in inulin space	+460	+35	..	−670	+490	0	+280
Days survival	4	4	3	4	6	3	4
	Dogs Given 0.9% NaCl Solution † (Group 2)						
Dog no.	28	29	30	31	32	38	40
Degree of vascular necrosis	0.5+	8+	6+	5+	5.5+	1+	5+
Maximum blood pressure	155	180	195	190	175	140	175
Maximum change in blood pressure	+40	+55	+45	+65	+40	+25	+55
Maximum change in Na^+	+ 5	+10	+10	+ 8	+14	+ 5	+ 5
Maximum change in K^+	+ 2.8	+ 3.9	+ 4.9	+ 1.6	+ 4.7	+ 1.9	..
Maximum change in Cl^-	+26	+14	+15	+ 4	+21	+12	+ 9
Change in blood volume	+291	−104	+77	..	+87	..	+46
Change in inulin space	+4050	+4010	+2370	..	+2460	..	+4125
Days survival	6	7	7	5	5	4	8
	Dogs Given Modified Locke's Solution † (Group 3a)						
Dog no.	43	44	45	46	47	50	53
Degree of vascular necrosis	11+	12+	8+	9+	10+	10+	10+
Maximum blood pressure	215	170	205	170	230	195	220
Maximum change in blood pressure	+100	+50	+80	+30	+95	+70	+85
Maximum change in Na^+	+ 3	+ 5	+ 2	+ 7	0	0	0
Maximum change in K^+	+ 2.5	+ 2.5	+ 3.0	+ 3.3	+ 2.0	+ 2.3	+ 1.9
Maximum change in Cl^-	+12	− 5	+ 5	− 3	+ 3	+ 3	+11
Change in blood volume	..	+18	+903	−210	+687	+325	−133
Change in inulin space	..	+1070	+2780	+4330	+3075	+2375	+3335
Days survival	5	4	6	4	9	10	6

* All electrolytes are reported in milliequivalents per liter.
† Groups 2 and 3a were given 0.9% NaCl and modified Locke's solution respectively, each at the rate of 100 ml./kg./day.

RESULTS

The changes in blood pressure and the development of lesions have been previously reported for bilaterally nephrectomized dogs given no fluid, for those given 0.9% NaCl, and for the first group of dogs given modified Locke's solution.[1] The bilaterally nephrectomized dogs given no fluid showed only very small changes in blood pressure, and two of the seven developed slight lesions. Those animals which received 0.9% NaCl solution developed moderate hypertension and lesions of moderate degree, whereas those given modified Locke's solution had the greatest increase in blood pressure and the most lesions (Table 1). The blood pressure has been previously presented in graphic form[1] and is summarized in Table 1 as maximum values.

Blood volume changes in the above three groups are also shown in Table 1. The nephrectomized dogs given no fluid had changes in blood volume ranging from an increase of 79 ml. to a decrease of 375 ml. (possible error). The dogs given 0.9% NaCl

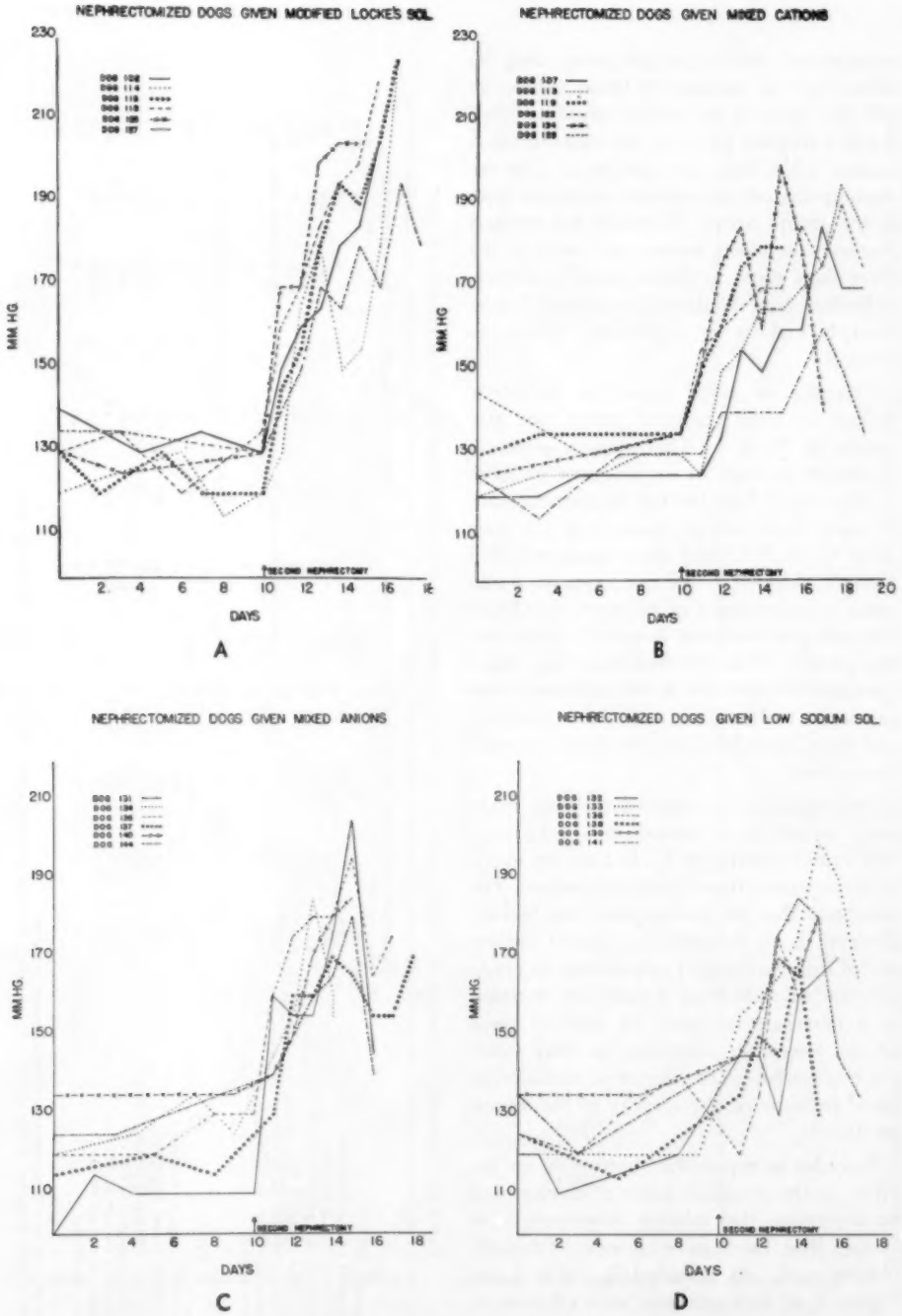

Fig. 1.—Blood pressure is plotted in millimeters Hg against time in days. The final 10 days of the control period as well as the experimental period (starting at second nephrectomy) are represented. The lines serve to facilitate tracing of changes in individual dogs rather than to indicate the blood pressure between the plotted points.

solution had similar changes except Dog 28, which had an increase in blood volume of 291 ml. Three of the animals given modified Locke's solution had large increases in blood volume (903, 687, and 325 ml.). The remaining animals had changes similar to those of the groups above. Although the greatest increases in blood volume are seen in the three dogs given modified Locke's solution, individual dogs developed pronounced hypertension without a significant change in volume.

Changes in inulin space in the three groups of dogs described above are also shown in Table 1. There was a slight to moderate increase in inulin space in three of the control dogs, but this was insignificant in comparison with the changes in the dogs given 0.9% NaCl and those given modified Locke's solution. The increase in inulin space is accompanied by an increase in blood pressure and vascular lesions in these last two groups. However, individual dogs show considerable variation in the amount of increase in inulin space and blood pressure, and these variations do not always parallel each other.

Determination of electrolytes was made every second day of the experimental period. The values reported in Table 1 are the maximum variations from the control values. The data show that the plasma potassium became elevated in all animals; the plasma sodium varied from no change to a moderate increase, and the chloride from a moderate decrease to a moderate increase. In none of these plasma electrolyte alterations is there clear-cut relationship to the degree of elevation in blood pressure or the severity of the lesions produced.

In order to repeat the observation on the effect of the modified Locke's solution and to determine the relative effectiveness of various ions, the dogs in Groups 3b through 6 were used. As indicated in Table 2 and Figure 1, all four solutions were effective in producing hypertension, but the modified Locke's solution is somewhat more effective than any of the others. The mixed anion

TABLE 2.—*Summary of Changes in Plasma Electrolytes, Blood Pressure, and Vessels in Bilaterally Nephrectomized Dogs**

	Dogs Given Modified Locke's Solution † (Group 3b)						Dogs Given Mixed Cation Solution † (Group 4)					
Dog no.	102	114	115	118	125	127	107	113	119	122	124	128
Degree of vascular necrosis	8+	8.5+	12+	10+	12+	11+	0	0+	10+	8+	8+	1.5+
Maximum blood pressure	210	225	225	220	205	195	185	195	180	190	200	160
Maximum change in blood pressure	+75	+100	+95	+85	+75	+60	+60	+65	+45	+55	+70	+30
Maximum change in Na^+	Lost	+ 2	− 2	− 2	+ 6	− 4	+ 3	0	+11	− 5	− 7	+11
Maximum change in K^+	Lost	+ 4.1	+ 6.7	+ 4.3	+ 2.5	+ 5.2	+ 6.2	+ 5.3	+ 4.4	+ 4.8	+ 5.1	+ 5.0
Maximum change in Cl^-	Lost	− 9	+11	0	− 3	+ 1	+17	+14	+19	+ 9	+25	+24
Days survival	7	7	8	7	6	8	10	9	6	10	7	11

	Dogs Given Mixed Anion Solution † (Group 5)						Dogs Given Low-Sodium Solution † (Group 6)					
Dog no.	131	134	135	137	140	144	130	132	133	136	138	141
Degree of vascular necrosis	11+	5+	10+	11+	7.5+	10+	4.5+	0.5+	11+	9+	8+	5+
Maximum blood pressure	205	185	185	170	185	180	170	170	205	175	170	185
Maximum change in blood pressure	+95	+60	+65	+50	+55	+55	+35	+45	+85	+55	+60	+50
Maximum change in Na^+	+ 2	+10	+ 7	+ 3	+ 3	+ 2	− 29	− 36	− 14	− 19	− 42	− 35
Maximum change in K^+	+ 3.8	+ 5.2	+ 2.8	+ 3.3	+ 2.4	+ 1.7	+ 2.8	+ 4.9	+ 2.0	+ 4.1	+ 4.3	+ 2.1
Maximum change in Cl^-	+ 4	+ 7	+ 7	− 4	+ 1	− 5	−31	−40	−10	−38	−36	−39
Days survival	6	5	8	8	7	6	5	6	7	4	7	7

* All electrolytes are reported in milliequivalents per liter.

† The solution indicated in the heading of each group was injected intraperitoneally at the rate of 100 ml./kg./day.

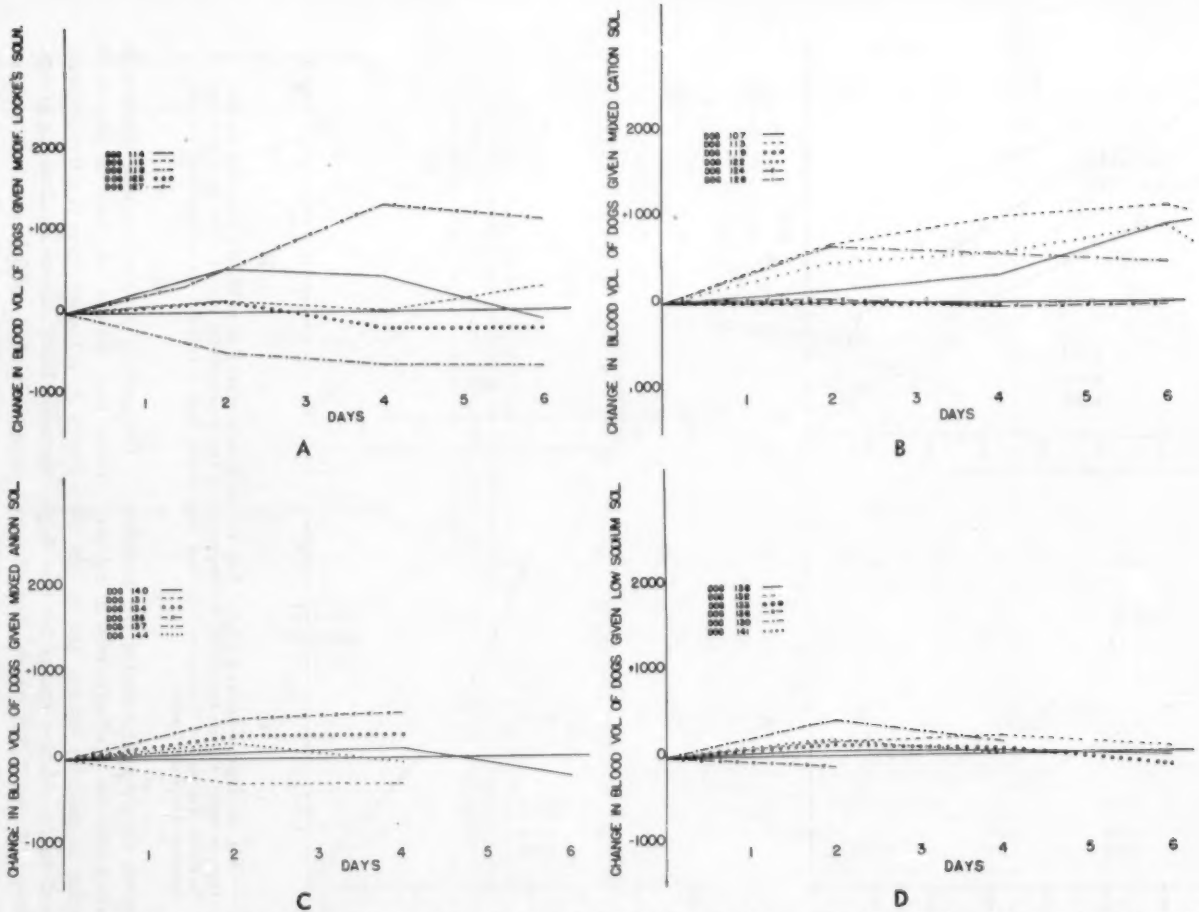

Fig. 2.—Change in blood volume in milliliters is plotted against time in days. Day 0 represents the beginning of the experimental period. The lines serve to facilitate tracing of changes in individual animals rather than to indicate the blood volume between the plotted points.

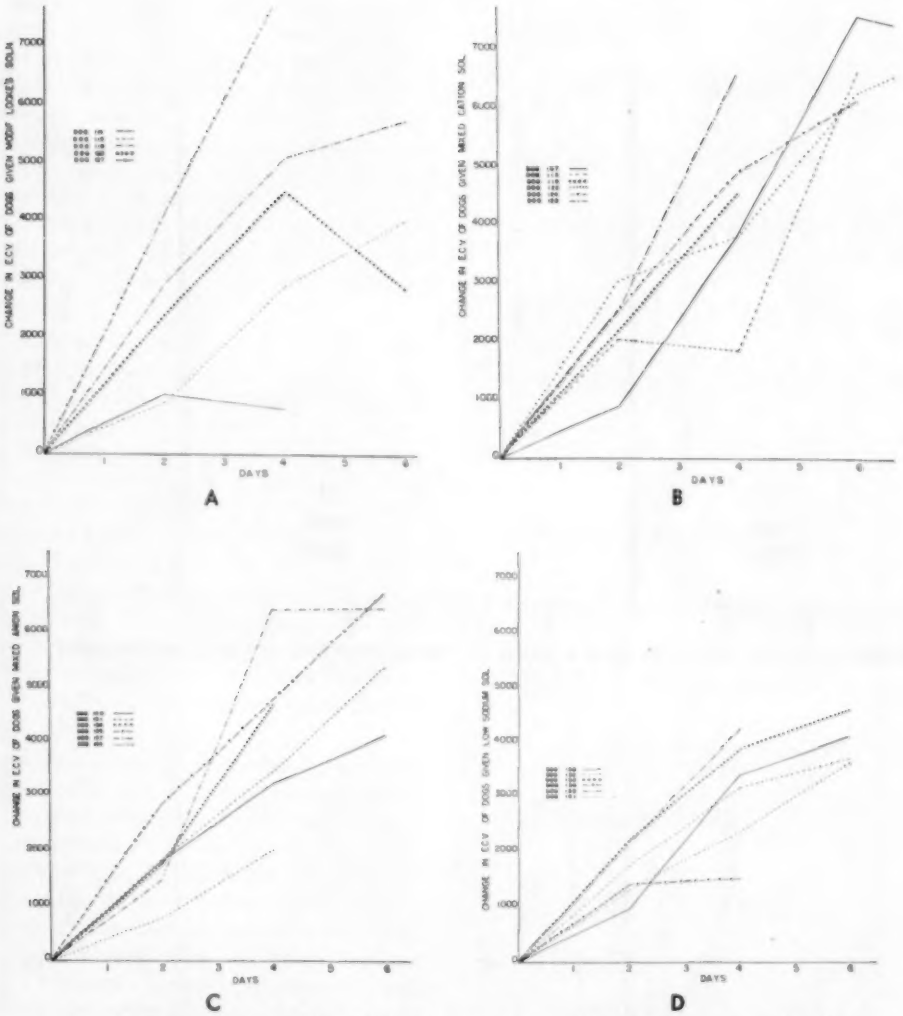

Fig. 3.—Change in extracellular fluid volume (inulin space) in milliliters is plotted against time in days. Day 0 represents the beginning of the experimental period. The lines serve to facilitate tracing the changes in individual dogs rather than to indicate the extracellular volume between the plotted points.

solution closely approximated the modified Locke's solution in effectiveness in the production of vascular lesions, and so did the mixed cation solution except for two dogs (Dogs 107 and 128), which showed very little response. Contrary to what might have been expected, the low-sodium solution was also moderately effective in the production of lesions.

Comparison of blood volume changes in these four groups of dogs is shown in Figure 2. Although there is a trend toward increased blood volume in all of the groups, many individual animals show no such increase. In Figure 3 the changes in inulin space for the same four groups of animals are shown. All of the animals show marked increases, but it should be pointed out that

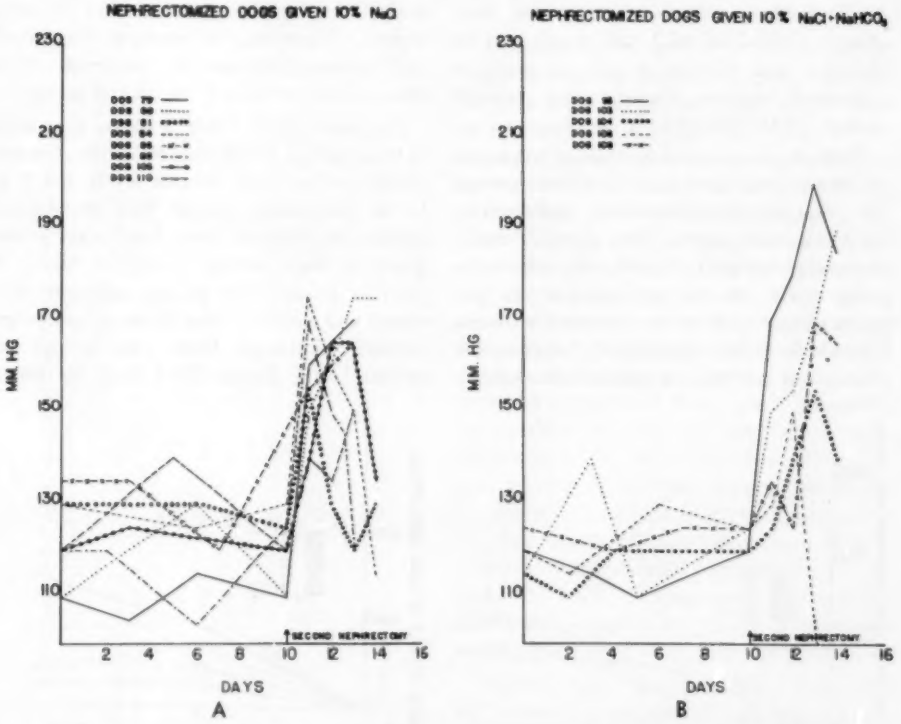

Fig. 4.—Blood pressure in millimeters Hg is plotted against time in days. The final 10 days of the control period as well as the experimental period (starting at second nephrectomy) are represented. The lines serve to facilitate tracing the changes in individual dogs rather than to indicate the blood pressure between the plotted points.

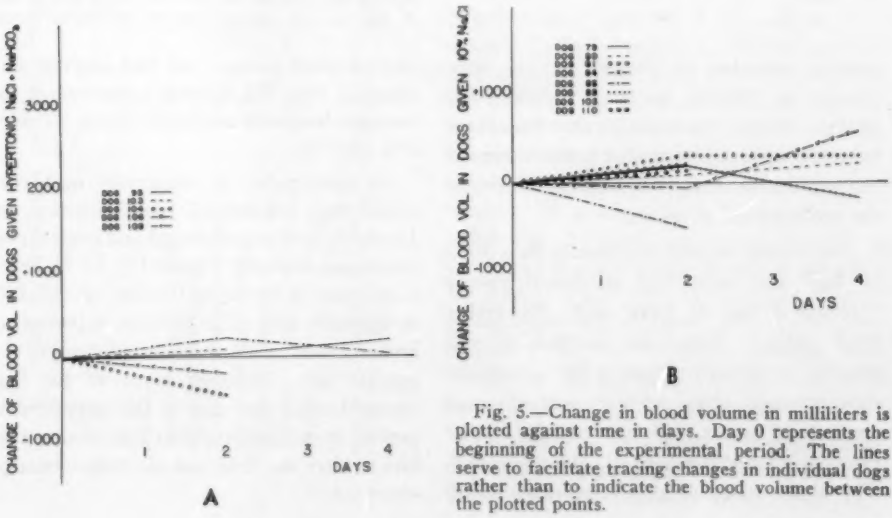

Fig. 5.—Change in blood volume in milliliters is plotted against time in days. Day 0 represents the beginning of the experimental period. The lines serve to facilitate tracing changes in individual dogs rather than to indicate the blood volume between the plotted points.

the individual animals showing the least change (1000-1500 ml.) had blood pressure elevation and vascular lesions as great as individual animals showing the greatest change (6000-7000 ml.).

Table 2 also presents the changes in plasma electrolyte concentrations. In all four groups the potassium was consistently increased to about the same degree. The plasma sodium changed prominently (decreased) only in the group given low-sodium solution. In the group given mixed cation solution the plasma chloride increased significantly, whereas the dogs given low-sodium solution developed a marked decrease in chloride. Very little change in chloride occurred in the other groups. Hence, it is apparent that these large changes in plasma electrolyte composition did not parallel the changes in blood pressure and the development of lesions.

In a further attempt to delineate the effects of fluid and ions, two additional groups (Groups 7 and 8) were used. By giving these animals hypertonic solution, it was possible to greatly increase the electrolyte concentration while adding only a small amount of water (64-100 cc/day). The changes in blood volume (Fig. 5) were small. The inulin space (Fig. 6) increased in all animals but one (a possible error in technique). However, the increase was small and in several animals was no greater than that in some animals of the control group.

Examination of Table 3 shows that both of these groups of dogs had markedly elevated plasma sodium and chloride levels, but it is to be particularly noted that the higher sodium and chloride levels are found in the group of dogs showing a slighter degree of vascular lesions. The plasma potassium elevation was similar to that in the other groups studied. Although these two groups of animals were shorter-lived than the other experimental groups and had slighter fluid changes, they did develop hypertension and vascular lesions of moderate degree (Table 3 and Fig. 4).

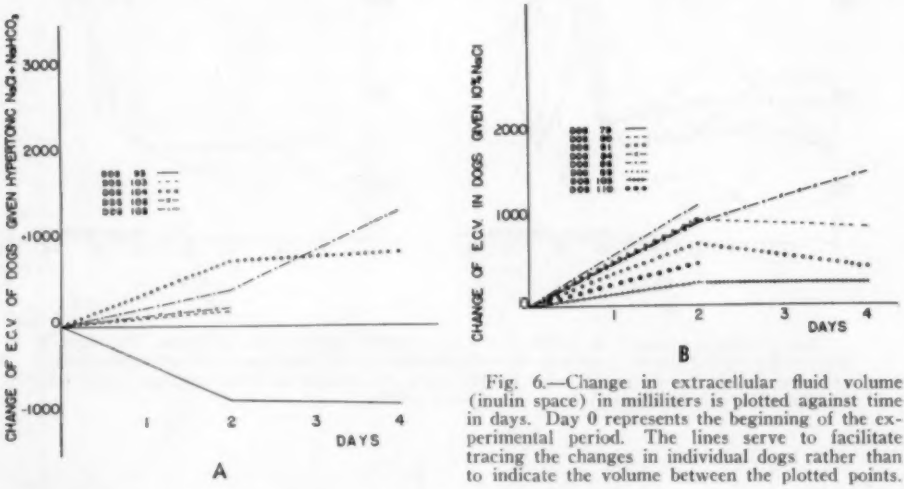

Fig. 6.—Change in extracellular fluid volume (inulin space) in milliliters is plotted against time in days. Day 0 represents the beginning of the experimental period. The lines serve to facilitate tracing the changes in individual dogs rather than to indicate the volume between the plotted points.

As anticipated in bilaterally nephrectomized dogs (Muirhead, Jones, Stirman, and Lesch [6]), both experimental and control dogs developed markedly elevated B. U. N. levels, a decrease in hematocrit values of 5%-10% in controls and 10%-20% in experimental animals, and a decrease in protein of 1-3 gm/100 ml. Although many of the dogs vomited near the end of the experimental period, it was believed that this occurred too late to alter the fluid and electrolyte changes observed.

TABLE 3.—*Summary of Changes in Plasma Electrolytes, Blood Pressure, and Vessels in Bilaterally Nephrectomized Dogs* *

	Dogs Given 10% NaCl Solution † (Group 7)							Dogs Given 10% NaCl and $NaHCO_3$ Solution † (Group 8)				
Dog no.	79	80	81	84	86	88	105	110	95	103	104	106	108
Degree of vascular necrosis	2.5+	0	0	0	0	0	1+	0	0.5+	7+	5.5+	0	2+
Maximum blood pressure	175	175	155	195	180	170	170	150	200	190	155	150	170
Maximum change in blood pressure	+65	+45	+30	+65	+50	+55	+50	+25	+85	+65	+30	+25	+45
Maximum change in Na^+	+45	+53	+45	+50	+48	+68	+57	+46	+18	+27	+54	+13	+98
Maximum change in K^+	+ 4.2	+ 3.4	+ 5.3	+ 6.7	+ 2.6	+ 5.7	+ 5.6	+ 2.6	+ 4.7	+ 0.7	+ 3.2	+ 2.6	+ 4.1
Maximum change in Cl^-	Lost	+30	Lost	+48	+38	+57	+57	+40	+ 8	+17	+20	+ 9	+15
Days survival	4	4	4	4	4	4	5	4	4	4	4	4	4

* All electrolytes are reported in milliequivalents per liter.
† The solution indicated in the heading of each group was injected intraperitoneally at the rate of 8.0 ml./kg./day.

COMMENT

Two strikingly constant features in all the experimental animals of this study were the increase in blood pressure and the increase in extracellular fluid (inulin space). This relationship of increased blood pressure to increased extracellular fluid corroborates the observations of Braun Menendez and von Euler [7] on nephrectomized rats and of Leonards and Heisler [8] and Houck [9] on nephrectomized dogs. In the experiments reported here, two mechanisms for increasing extracellular fluid were used. Groups 2 through 6 were given large amounts of isotonic or hypotonic fluid, thus increasing extracellular fluid by the addition of exogenous fluid. On the other hand, Groups 7 and 8 were given small amounts of hypertonic solution, and the increase in extracellular fluid apparently resulted from a shift of water from intracellular to extracellular position. In both circumstances hypertension developed, although the means of increasing extracellular fluid were quite different.

Since several techniques have been used by various investigators to increase extracellular fluid and all have been consistently accompanied by an increased blood pressure, it would appear that the nephrectomized animal reacts characteristically to increased extracellular fluid by the development of hypertension. That this is not a simple relationship between increased extracellular fluid and increased blood pressure is indicated by the fact that experimental procedures such as chronic pericarditis and stenosis of the inferior vena cava are followed by increased extracellular fluid without associated hypertension. It seems reasonable, therefore, to stress that the nephrectomy is probably a factor in the reaction to increased extracellular fluid. Furthermore, the fact that animals given hypertonic solutions developed hypertension with increases in extracellular fluid similar in degree to the controls suggests that electrolyte also plays a role.

The effect of blood volume in the production of elevated blood pressure and vascular disease was also studied. Byrom and Dod-

son [10] reported vascular lesions resulting from a rapid increase in blood volume, but Johnston and Moore [11] were unable to corroborate their observations. In the series of experiments reported in the present report, change in blood volume was determined in 48 dogs during the period that they developed hypertension. Since 24 of the 48 had increases in blood volume of 100 ml. or less, increase in blood volume does not appear to be a critical factor in the development of the increased blood pressure or the vascular disease.

The determination of plasma concentrations of electrolytes (Na, K, and Cl) was disappointing in that there was no parallel between change in plasma concentrations of electrolyte and the changes in blood pressure or vascular disease. The potassium level increased to approximately the same degree in both controls and experimental animals, thereby failing to accompany the variation in incidence of blood pressure and vascular lesion. Contrariwise, the plasma Na and Cl were found to vary widely in Groups 2, 4, 6, 7, and 8 without accompanying consistent alteration in blood pressure or vascular disease. In fact, it should be pointed out that the greatest and most constant change in blood pressure and vascular disease occurred in the dogs given modified Locke's solution where no appreciable changes in Na and Cl occur.

Although the concentrations of plasma electrolytes failed to correlate with changes in blood pressure and vascular disease, the importance of electrolytes cannot be dismissed. When attention is focused on the ionic composition of the administered fluid, it is apparent that animals receiving the same amount of fluid of different ionic composition developed different degrees of hypertension and vascular disease. The modified Locke's solution induced greater elevation in blood pressure and more consistent vascular disease than any of the other solutions used. The mixed cation and mixed anion solutions did not result in clear differences in the effectiveness of these two types of ions.

Further comparison of the effects of the solutions used indicates that the mixed anion is more effective in producing vascular lesion than the 0.9% NaCl, and the hypertonic $NaCl \cdot NaHCO_3$ solution is more effective than the hypertonic NaCl, even though there is no apparent difference in their effect on blood pressure. The fact that these solutions vary from one another by their anionic composition indicates that the anion may be of significance in the production of vascular lesions and should have careful consideration in future studies of hypertensive vascular disease. Whether this effect is a direct action by a specific ion or whether it is the effect of the anion on acid-base equilibrium must await further work.

The low-sodium solution was used principally to test the importance of the sodium ion under these experimental conditions. The importance of this ion in some types of experimental hypertension is well documented. The potentiating effect on desoxycorticosterone-acetate-induced hypertension reported by Selye and Pentz,[12] the production of hypertension by oral administration of hypertonic NaCl reported by Selye and Stone,[13] and the potentiating effect of renin- and cortisone-induced hypertension reported by Masson, Plahl, Corcoran, and Page,[14] as well as the alterations in Na metabolism accompanying essential hypertension by Green, Wedell, Wald, and Learned,[15] all indicate the importance of this ion. Yet it was possible to induce hypertension and vascular disease in these animals, even though the administered fluid was very low in sodium and caused a marked reduction in the plasma sodium levels. The significance of this observation is not clear. It may be that the importance of the Na ion in the induction of hypertension is related to its property of inducing fluid retention or altered fluid distribution. If fluid retention or altered distribution is induced by some other means (in this instance by overloading nephrectomized dogs with fluid), this action of sodium may not be of significance.

The fact that the administration of different electrolyte solutions induced different degrees of hypertension and vascular disease

and the fact that plasma electrolyte levels failed to reflect this relationship indicate that other techniques must be used if we are to gain further understanding. Some indication of the type of technique that may be productive in this situation has been reported by Tobian and Binion.[16] By determining the electrolyte concentrations in the aortic wall of hypertensive animals, these workers found changes that were not apparent in other organs. It may be that the true significance of electrolyte alterations will become apparent only as such studies are pursued further.

Finally, the opinion expressed in our first paper should be repeated: The absence of the kidneys in these experiments rules out the presence of a renal pressor substance as a factor in the hypertension reported here. Kolff, Page, and Corcoran[17] have made studies with animals made hypertensive by this technique and have obtained evidence that supports the above opinion. Furthermore, the rapidity with which hypertension develops under these conditions casts doubt on the hypothesis that hypertension results from the accumulation of a pressor substance normally inactivated or excreted by the intact kidney. Kolff and Page[18] have shown, however, that the kidney cannot be excluded from consideration, even though it has been removed. They have transplanted kidneys from normal dogs into the carotid and jugular vessels of a dog made hypertensive by the technique reported here and have found that blood pressure returns to normal in about two hours. Transplantation of other organs had no such effect. It may be, therefore, that the kidney normally stabilizes fluid and electrolyte distribution throughout the body by some mechanism as yet unknown. If such a stabilizing mechanism were lost (by nephrectomy), the introduction of a fluid load might lead to alterations in fluid and electrolyte distribution that would not otherwise occur. Under such circumstances the vascular system might be placed under a stress that would lead to hypertension and vascular disease.

CONCLUSIONS

1. Hypertension and arterial necrosis are produced within very short periods of time in bilaterally nephrectomized dogs by intraperitoneal injection of a variety of electrolyte solutions.

2. The increased extracellular fluid induced by this technique is consistently accompanied by hypertension, but no such relationship between blood volume and hypertension is apparent.

3. The electrolyte composition of the fluid appears to be a factor in the degree of hypertension and vascular disease induced, and the anionic composition may be of particular significance in the development of the vascular disease.

REFERENCES

1. Orbison, J. L.; Christian, C. L., and Peters, E.: Studies on Experimental Hypertension and Cardiovascular Disease: I. Method for the Rapid Production of Malignant Hypertension in Bilaterally Nephrectomized Dogs, A. M. A. Arch. Path. **54**:185, 1952.

2. Skeggs, L. T., Jr., and Leonards, J. R.: Studies on the Artificial Kidney: 1. Preliminary Results with a New Type of Continuous Dialyzer, Science **108**:212, 1948.

3. Kolmer, J. A., and Boerner, F.: Approved Laboratory Technique, Ed. 4, New York, Appleton-Century-Crofts, Inc., 1945, p. 818.

4. Chinard, F. P.: Estimation of Plasma Volume by Dye Dilution Method, Methods M. Res. **4**:38, 1951.

5. Higashi, A., and Peters, L.: Rapid Colorimetric Method for the Determination of Inulin in Plasma and Urine, J. Lab. & Clin. Med. **35**:475, 1950.

6. Muirhead, E. E.; Jones, F.; Stirman, J. A., and Lesch, W.: Hemolytic Anemia of the Dog Following Bilateral Nephrectomy, Am. J. Physiol. **172**:371, 1953.

7. Braun Menendez, E., and von Euler, U. S.: Hypertension After Bilateral Nephrectomy in Rat, Nature, London **160**:905, 1947.

8. Leonards, J. R., and Heisler, C. R.: Blood Pressure of Bilaterally Nephrectomized Dogs, Fed. Proc. **11**:247, 1952.

9. Houck, C. R.: Effect of Hydration and Dehydration on Hypertension in the Chronic Bilaterally Nephrectomized Dog, Am. J. Physiol. **176**:183, 1954

10. Byrom, F. B., and Dodson, L. F.: Causation of Acute Arterial Necrosis in Hypertensive Disease, J. Path. & Bact. **60**:357, 1948.

11. Johnston, D. G., and Moore, R. D.: Acute Arterial Plethora, Proc. Soc. Exper. Biol. & Med. **79**:138, 1952.

12. Selye, H., and Pentz, E. I.: Pathogenetical Correlations Between Periarteritis Nodosa, Renal Hypertension and Rheumatic Lesions, Canad. M. A. J. **49**:264, 1943.

13. Selye, H., and Stone, H.: Role of Sodium Chloride in Production of Nephrosclerosis by Steroids, Proc. Soc. Exper. Biol. & Med. **52**:190, 1943.

14. Masson, G. M. C.; Plahl, G.; Corcoran, A. C., and Page, I. H.: Accelerated Hypertensive Vascular Disease from Saline and Renin in Nephrectomized Dogs, A. M. A. Arch. Path. **55**:85, 1953.

15. Green, D. M.; Wedell, H. G.; Wald, M. H., and Learned, B.: Relation of Water and Sodium Excretion to Blood Pressure in Human Subjects, Circulation **6**:919, 1952.

16. Tobian, L., Jr., and Binion, J.: Tissue Electrolytes in Renal and DCA Hypertension, J. Clin. Invest. **32**:608, 1953.

17. Kolff, W. J.; Page, I. H., and Corcoran, A. C.: Pathogenesis of Renoprival Cardiovascular Disease in Dogs, Am. J. Physiol. **178**:237, 1954.

18. Kolff, W. J., and Page, I. H.: Blood Pressure Reducing Function of the Kidney: Reduction of Renoprival Hypertension by Kidney Perfusion, Am. J. Physiol. **178**:75, 1954.

Atherosclerosis

IV. The Relation of the Composition of the Blood Lipids to Atherosclerosis in Experimental Hyperlipemia

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The quantitative estimation of the lipids and of their fractions by chemical methods is the accepted analytical procedure and that by which other methods of lipid analysis are standardized. The quantitative separation of a lipid extract into fractions may seem to be approximated only, because the fractions themselves are mixtures. This is true especially of the neutral fat and of the phospholipid fractions, in which several fatty acids may participate in the composition of the compounds. The chemical methods generally prescribed for lipid analysis with separation of the fractions require fairly large amounts of extract, which in turn demand considerable original tissue material. Studies of the total lipids of the blood and of their fractional distribution in health and disease have been thwarted previously because methods for making these determinations with small amounts of serum had not been devised. The exception to this statement, of course, is the cholesterol of the blood. The systems first devised for the colorimetric estimation (microanalysis) of the constituents of the blood in small samples included directions for measuring the cholesterol content. Since then, countless reports on the cholesterol content of the blood have been published.

Fluctuations in the amount of cholesterol in the blood are considered significant in various disorders. Some investigators and clinicians regard the cholesterol value as an index for the level of all of the other lipid fractions of the blood. This opinion rests upon a certain amount of presumption, because parallel quantitative determinations of the other fractions of the total lipids of the blood generally have not been investigated and, when this has been done, variations have been observed, especially of the neutral fat fraction, independent of the cholesterol values. Or, variations in all fractions have been noted, more in one than in the others.

The lipids of the blood are transported in the plasma as emulsified particles of mixed chemical composition, suspended in an aqueous medium containing organic and inorganic solutes. Thus, there are two systems in the blood—the one, the lipid suspension, the other, the aqueous—in which the lipid is emulsified. Each system has a solvent in which solutes are dissolved. For practical purposes the lipids of the blood consist of three main classes: (1) the neutral fats, namely, the triglycerides of the saturated and the unsaturated fatty acids; (2) the phosphatides, such as the cerebrosides and the ceramides, which are closely related in chemical structure to the neutral fats, and (3) the sterols, cholesterol and its esters. The neutral fats are mixed triglycerides, and each triglyceride usually is constituted chemically according to the simple principle proposed by Hilditch.[1] According to this principle, as soon as a given fatty acid forms about 35% of the total fatty acids in a fat,

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it will occur at least once in every triglyceride molecule, and no simple triglycerides containing three groups of the same fatty acid will occur until such an acid forms nearly two-thirds or more of the total fatty acids. Each fatty acid accordingly competes independently of the others for union with glycerin. The formula fails to describe accurately the general triglyceride structure of animal fats in only two classes: (1) animal depot fats unusually rich in mixed glycerides containing stearic groups and (2) milk fats, in which the lower saturated acids from butyric to lauric occur in appreciable amounts (13%).

The monoaminomonophosphatides, lecithin and cephalin, are choline and cholamine phosphoric acid esters which have replaced, respectively, one of the three fatty acid radicals of a triglyceride fat. These diglycerol esters, lecithin and cephalin, thus are compounds chemically similar to neutral fats. According to Thannhauser, 70% to 90% of the total phospholipids of the serum may be evaluated as lecithin. Cholesterol, the significant sterol of the blood, is chemically a monatomic alcohol, solid at body temperature. It combines with a single fatty acid to form an ester compound. The cholesterol esters containing the higher saturated fatty acids, such as stearic, are solid at body temperature; those of the lower unsaturated fatty acids (oleic) are liquid or semiliquid. Cholesterol esters form normally 70% to 75% of the total serum cholesterol.

The esterified fatty acids occur in the chemical composition of all lipid fractions except the free cholesterol. The fatty acids in both the phospholipid and the cholesterol ester fractions probably follow the rule of Hilditch as applied to neutral fats. Accordingly, in the presence of a large percentage of the neutral fats liquid at body temperature, similar fatty acids will occur in the phospholipid and in the cholesterol esters; whereas in the presence of a high percentage of the solid neutral fats these fatty acids will appear also in the phospholipid and cholesterol ester fractions. These compounds, however, are solid at body temperature and require a fat solvent for solution. Thus, fatty acids are significant integral constituents of the three main fractions of the blood lipids, free cholesterol being the only exception.

The general practice of measuring the cholesterol content of the blood in a large measure can be related to the ease with which its value is determined with small amounts of blood. Moreover, great emphasis has been given to the cholesterol content of the blood in the evolution of atherosclerosis. This focused interest in the cholesterol content of the serum in various disorders has occurred without realization that the proportion of this fraction does not always parallel the neutral fat and the phospholipid fractions. Bauer and Hirsch,[2] in 1949, published a method for the colorimetric estimation of the total esterified fatty acids in small amounts of serum. Later this method was reevaluated, modified slightly, and more closely standardized.[3] This method has been used in evaluating the esterified fatty acids in the blood lipids of normal human adults,[4] of infants and children,[5] of patients with diabetes,* of children with fibrocystic disease of the pancreas,[5] and of rabbits with experimentally produced disorders.† These studies have disclosed that the amount of the esterified fatty acids and their distribution in the three main classes of the blood lipids may vary greatly from the normal fasting level. They also have demonstrated that the quantitative distribution of the esterified fatty acids in the three main lipid fractions of the blood are significant data in the evaluation of hyperlipemic disorders.

In a previous report [11] the composition of the emulsified lipids in the blood at the fasting level and in the various forms of hyperlipemia was likened to a solvent and its solutes, the proportions of which can vary. The fats liquid at body temperature, such as olein, are the solvents for those solid at body temperature. At the one extreme, in some lipemias, the solvent lipids are in excess, as with simple postprandial hyper-

* References 6 and 7.

† References 8, 9, and 10.

lipemia, the early phases of idiopathic hyperlipemia, most forms of diabetic hyperlipemia, and some of the lipemias associated with nephritis in the human (nephrosis) or produced experimentally in animals. At the other extreme, the solutes approach the supersaturation level, as in the experimental feeding of cholesterol to rabbits and other animals, with the production of atherosclerosis, and in diabetics and other patients with xanthomatosis. Persistence of the imbalance when the lipid solute is in excess occurs in rabbits given food enriched with cholesterol, and lipids containing large amounts of cholesterol are deposited in the lining of the aorta and in many other tissues of the body.

In a previous report[11] we investigated the effects of feeding lipids containing a large content of the solvent liquid fats on the lipids of the blood and other tissues in the rabbit, the test animal used in many studies on the experimental production of atherosclerosis. Rabbits received a balanced unrestricted diet supplemented daily by 15 to 50 cc. of emulsified fat (cream) given by gavage. The cream has a high content of solvent fats, especially olein, and contains about 36% fat with a mixed composition as follows:

	Per Cent
Butyric acid	2.9
Caproic acid	1.9
Caprylic acid	0.8
Capric acid	1.6
Lauric acid	5.8
Myristic acid	19.8
Palmitic acid	15.2
Stearic acid	15.0
Oleic acid	31.9
	94.9

Cream contains 0.1% total cholesterol and 0.3% to 0.4% phospholipids.

Rabbits fed thus became hyperlipemic in a week or 10 days. The neutral fats, the phospholipids, and the cholesterol fractions participated in the lipid increase during this phase of hyperlipemia (Fig. 1). As the daily feeding of cream continued, especially with larger quantities, the hyperlipemia increased, and at these higher levels the neutral fat fraction increased more than the others. When the esterified fatty acids of the blood exceeded appreciably 20 to 30 mEq/liter, the rabbits had an acute metabolic disturbance, with diarrhea; sudden marked rise of the total esterified fatty acids of the blood, chiefly of the neutral fat fraction; hyperglycemia, and azotemia, and they died. The livers of these animals had marked fatty changes. There were no lipid deposits in the aorta and other tissues. This acute metabolic disorder with hyperlipemia, in which the lipids of the blood have a high neutral fat content, contrasts with the chronic wasting disorder observed in rabbits fed cholesterol. These cholesterol-fed rabbits also become hyperlipemic, but the lipids of the blood have a disproportionately high content of free cholesterol and cholesterol esters.

The solvents in the emulsified lipids of the blood are those liquid at body temperature, chiefly the neutral fats (olein). The lipids dissolved in this medium are those solid at body temperature, such as cholesterol, certain of its esters, and other lipid compounds such as palmitin and stearin. We found that the blood lipids in the hyperlipemia produced in rabbits with a cream diet have a large content of neutral fat (solvent) and probably reflect in composition the lipids of the cream fed. Lipids of the blood in the hyperlipemia of this composition are not deposited in the lining of the blood vessels and in the reticuloendothelial tissues of the animal. As stated, the livers of these rabbits had marked fatty changes. However, with the hyperlipemia produced in rabbits fed cholesterol and where the lipids have a high content of solute (cholesterol and its esters), lipids of this composition are deposited in the reticuloendothelial tissues of the body and atheromas containing lipophages laden with lipids appear in the lining of the aorta and the arteries. The differences in the effects of these chemically dissimilar hyperlipemias on the reticuloendothelial and other tissues of the rabbit stand in sharp contrast. It is possible also

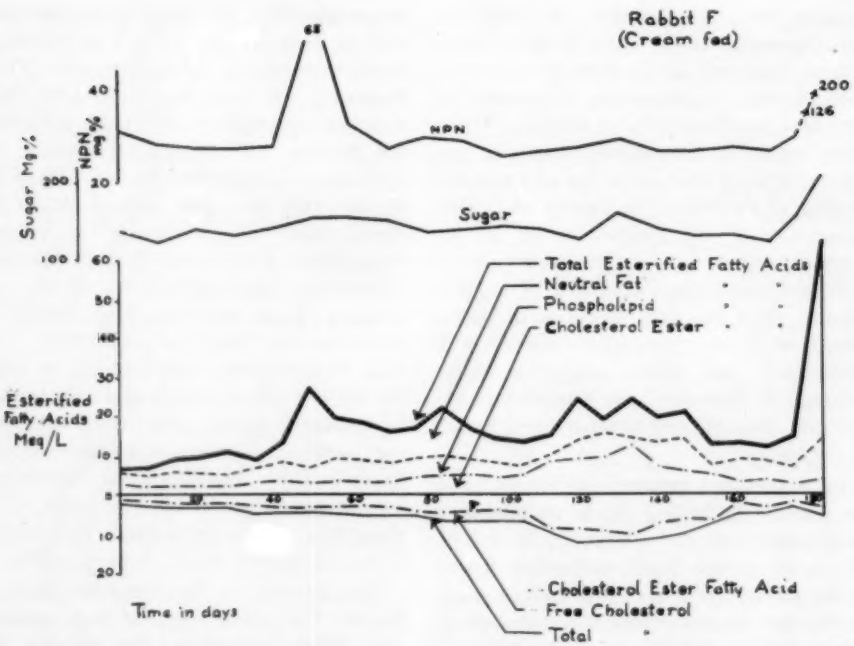

Fig. 1.—Graphs illustrating the composition of the lipids of the blood in the hyperlipemia of rabbits with a diet supplemented by cream.

that lipids deposited in the proportions prevailing in the circulating blood lipids, after deposition in tissues, become disproportionate by utilization of the solvent lipids faster than the solute lipids or by an accumulation of the solutes. The lipid mixture thus acquires the composition which is capable of stimulating tissue reactions.

The relationship between the hypercholesteremia and atherosclerosis of the aorta produced experimentally in rabbits and other animals with diets to which cholesterol has been added is well established. Although hypercholesteremia in rabbits fed cholesterol always preceded the deposition in the tissues of cholesterol, Weinhouse and Hirsch [12] observed no direct correlation between the height or duration of the hypercholesteremia and the severity of the atherosclerosis. However, these studies were made when a method had not been developed for determining the separate lipid fractions in small amounts of blood, by which frequent estimations of the fractional distribution of the lipids in the hyperlipemia of each animal could be made. Such serial analysis could disclose significant variations in the composition of the blood lipids from the initial normal and thus afford data for a possible correlation of the composition of the lipids in the experimentally produced hyperlipemia and the occurrence of the atherosclerosis in rabbits fed cholesterol.

Hypercholesteremia occurs regularly in the evolution of experimental atherosclerosis in animals and is considered a primary requisite. The tissues of the rabbit or of other animals chosen for the experimental feeding are initially believed to be normal. Yet as hyperlipemia develops, atherosclerotic lesions appear in the aorta and lipid deposits occur in other tissues, chiefly the reticuloendothelial, of the body. The experiments actually should be considered a study of the relation or role of a hyperlipemia to the evolution of atherosclerosis in the tissues

of a basically normal animal. The atherosclerotic lesions in the aorta appearing in these animals are not distributed diffusely in the lining, but rather are focal deposits in the aortic arch, along the posterior wall of the descending aorta, about the ostiums of the intercostal arteries, and at the bifurcation of the aorta into the iliac arteries. Similar loci of distribution are observed in atherosclerosis of the aorta in the human. Accordingly, there is rather clear evidence, in the animals used for experimental purposes and in the human, that structural tissue factors are concerned with the actual deposition of the lipids circulating in the blood. Thus, the hyperlipemia with the lipid composition characteristics which occurs experimentally when animals are fed cholesterol becomes the dominant factor in the production of atherosclerosis, but associated with this is a structural factor of the arterial tissues which determines the sites of deposition. Aortic and arterial tissues, structurally constituted or modified by whatever agents and rendered susceptible, thus become loci for lipid deposits, the initial phase of atherosclerosis.

Hyperlipemia is important in the evolution of experimental atherosclerosis, but much more significant can be the fractional content of the lipids in this hyperlipemia, as the experiments in cream-fed rabbits disclosed. In contrast with the hyperlipemia of cream-fed rabbits, the experimental hyperlipemia of cholesterol-fed animals, with its disproportionately high content of cholesterol and its esters, is associated with focal deposits in aortas which initially are not diseased but apparently have structurally susceptible loci. Factors in tissues rendering them susceptible thus are significant in the actual deposition of the lipids circulating in the blood.

The lipids circulating in the blood plasma are an emulsion distributed in particles that vary in size.[10] Under ordinary conditions these particles are small and constitute the so-called "blood dust." In hyperlipemia the emulsion includes particles of larger dimension, readily visible under the microscope and giving to such serums an opalescence.

Because the composition of the lipids of the blood in hyperlipemia seems to be significant in the evolution of experimental atherosclerosis in rabbits, further investigations of this hyperlipemia factor in rabbits were made. Due regard must be given to the relatively short duration of most of the experiments on atherosclerosis in animals. Speculation on the influence of a much longer time can be presumptuous, because of uncontrollable variables.

The methods for blood lipid analysis are the same as those published in a previous report,[11] except for a slight modification of the method for cholesterol, which gave comparable results. Four rabbits were fed cream with 1% cholesterol. This is a saturated solution, with an excess of the cholesterol in the cream at room temperature. These rabbits became hyperlipemic to a level considerably above that observed in rabbits fed cream only. All fractions of the blood lipids participated in this increase, the free cholesterol and the cholesterol esters gradually exceeding the increase of the other fractions, although there were some variations in this respect. The acute metabolic death observed in rabbits fed only cream occurred also among these animals, and when it happened the neutral fat fraction of the blood lipids rose disproportionately to a high level. Small atheromas were found in the arch and about the openings of the intercostal and other arterial branches of the aorta in Rabbit 2 and Rabbit 5, fed for 154 and 140 days, respectively. Rabbit 1 and Rabbit 4, fed for 55 and 120 days, had no lesions.

The graphs in Figures 4-6 inclusive illustrate the evolution of the hyperlipemia and the distribution of the blood lipids in the neutral fat, the phospholipid, and the esterified and free cholesterol fractions in three of the four rabbits given daily by gavage 25 to 50 cc. of cream containing 1% cholesterol supplemental to a regular balanced chow diet. The graphs illustrate the progressive increase in the level of the hyperlipemia and demonstrate that all fractions participate roughly proportionately in this increase ex-

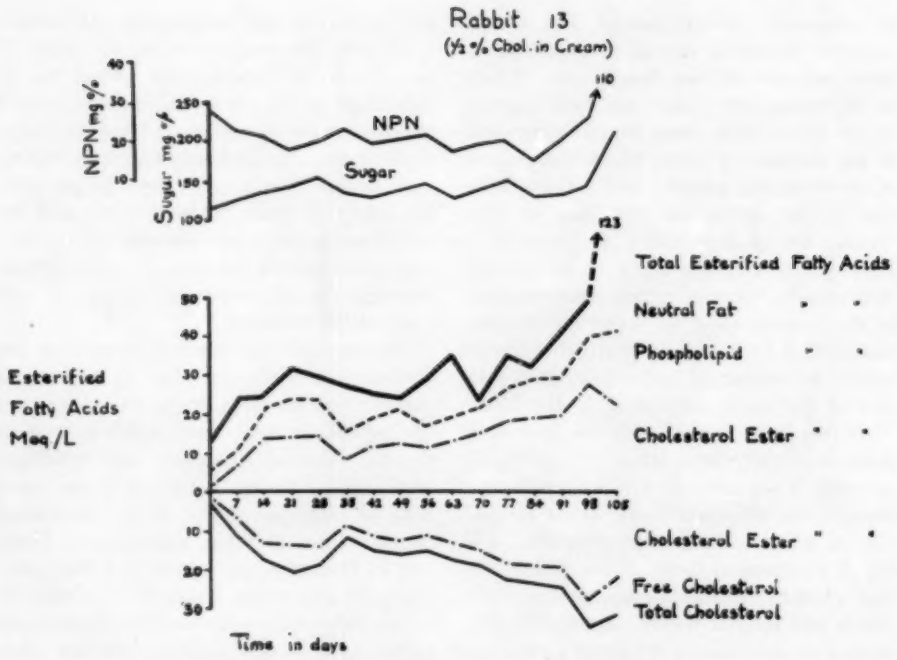

Fig. 2.--Graphs illustrating the composition of the lipids of the blood in the hyperlipemia in rabbits with a diet supplemented by cream containing 0.5% cholesterol.

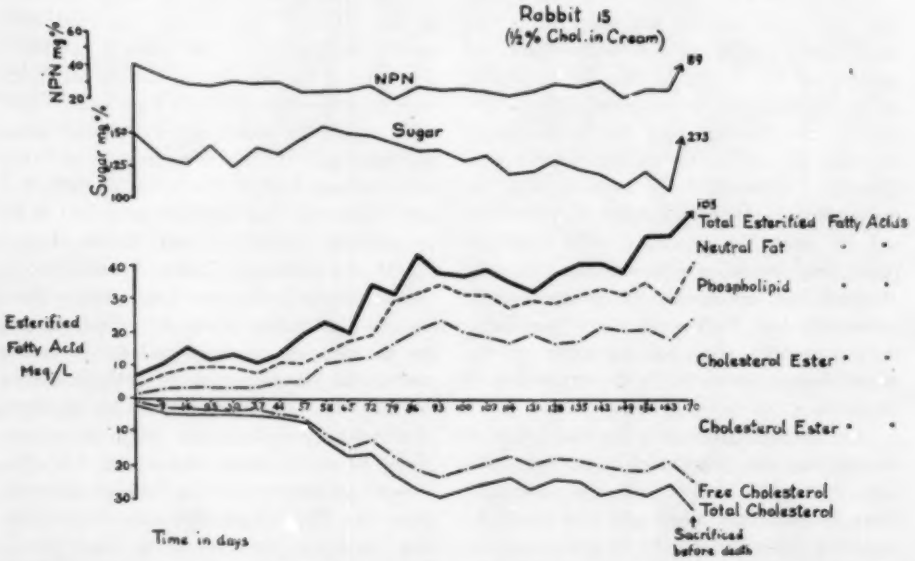

Fig. 3.—Graphs illustrating the composition of the lipids of the blood in the hyperlipemia in rabbits with a diet supplemented by cream containing 0.5% cholesterol.

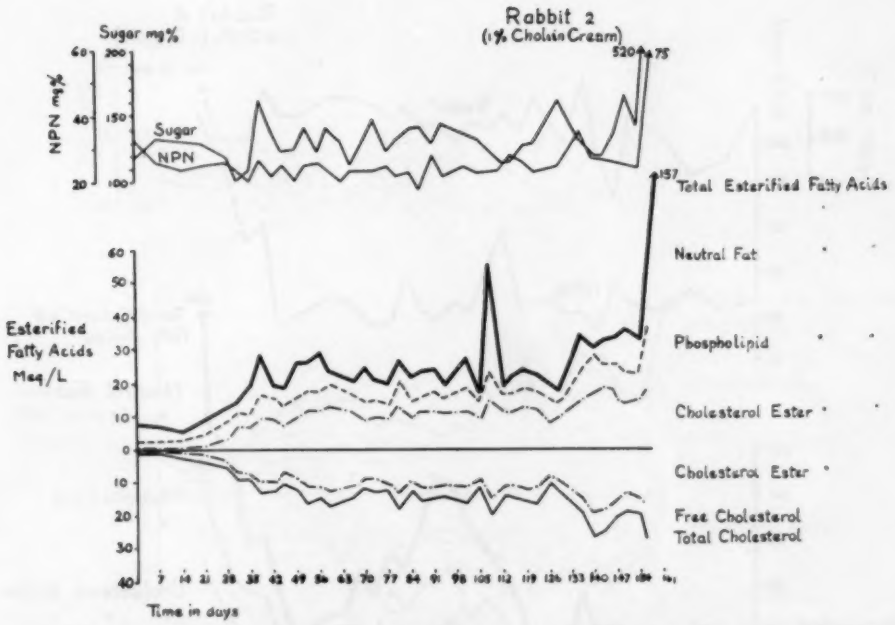

Fig. 4.—Graphs illustrating the composition of the lipids of the blood in the hyperlipemia in rabbits with a diet supplemented by cream containing 1% cholesterol.

cept for those abrupt elevations where the neutral fat increased more than the others, especially in an acute toxic phase. Rabbits 2 and 5, fed for 150 and 140 days, respectively, had several small plaques in the lining of the arch of the aorta, around the ostium of intercostal arteries, or elsewhere. The liver of each rabbit had marked fatty changes. The cytoplasm of the liver cells in Rabbits 1 and 4 had large droplets of lipids; the cytoplasm of the liver cells in Rabbits 2 and 5 had many small droplets. A few mononuclear phagocytes in other tissues had small globules of lipid. None of these rabbits fed cream with 1% cholesterol, even the two with small plaques in the aorta, had the lipid saturation of tissues which occurs in rabbits fed large quantities of cholesterol without cream. Two others, Rabbits 13 and 14, received cream with 0.5% cholesterol for 105 and 170 days, respectively. The graphs in Figures 2 and 3 illustrate the evolution of the hyperlipemia in these animals and the increase in the fractions of the blood lipids as this progressed. All fractions of the blood lipids participated in the total increase, and the values plotted formed graphs comparable to, if not fully identical with, those expressing the values obtained in rabbits fed cream with 1% cholesterol.

Many reports have been published on the experimental production of atherosclerosis in rabbits and other animals by feeding cholesterol.[18] Hyperlipemia is accepted as a significant factor in the evolution of the aortic and arterial deposits. However, few studies have included more than brief comments on the composition of the blood lipids in the hyperlipemic stage. The composition of the blood lipids in the hyperlipemia of experimental atherosclerosis deserves investigation, because such analyses may afford significant data in relating the chemical composition of the lipids of the blood to their deposition in the lining of blood vessels. For comparison with the composition of the hyperlipemia of rabbits fed 1% and 0.5%

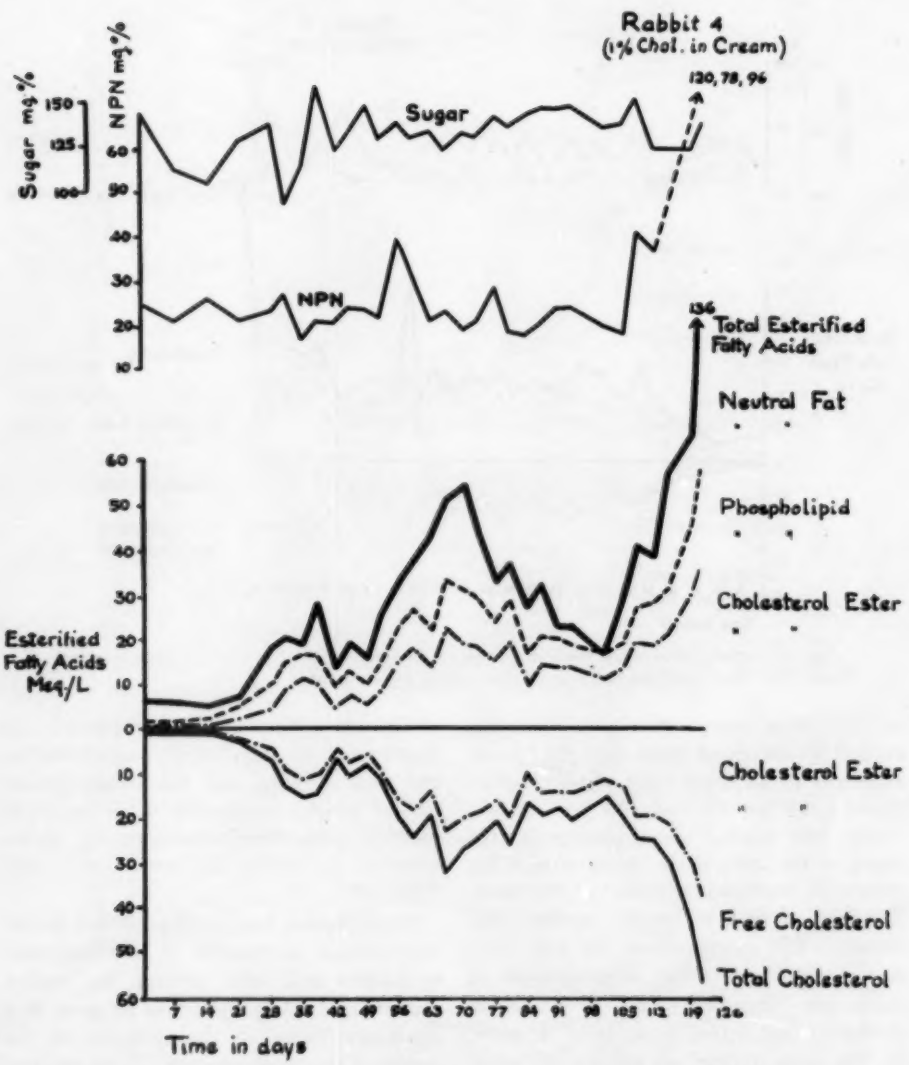

Fig. 5.—Graphs illustrating the composition of the lipids of the blood in the hyperlipemia in rabbits with a diet supplemented by cream containing 1% cholesterol.

cholesterol in cream, other rabbits were fed cholesterol without cream. These rabbits received a standard chow ration over which an ether solution of cholesterol was poured and the solvent evaporated. The amounts of cholesterol added to the diet in this way were moderate, not excessive. The evolution of the hyperlipemia in these cholesterol-fed rabbits and the distribution of the neutral fat, phospholipids, cholesterol esters, and free cholesterol are plotted in the graphs of Figures 7 and 8. Within two weeks the total lipids increased abruptly 7- to 10-fold, with the maximum rise in the cholesterol ester fraction. At the peak level the cholesterol fraction in one rabbit had increased to 45 times the initial value; the phospholipids to 9 times, and the neutral fat, to 4.5 times.

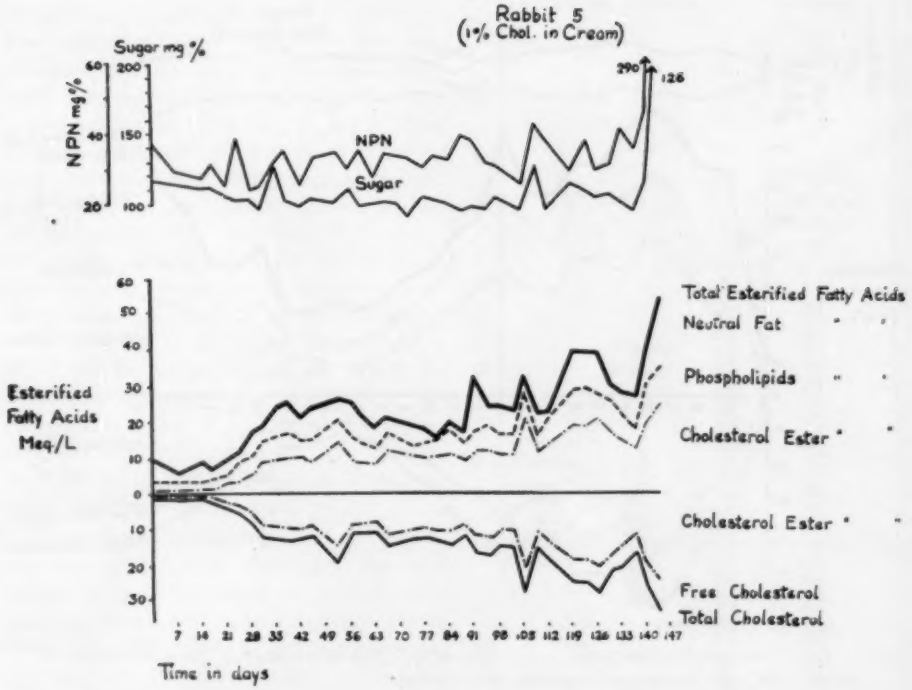

Fig. 6.—Graphs illustrating the composition of the lipids of the blood in the hyperlipemia in rabbits with a diet supplemented by cream containing 1% cholesterol.

As the feeding continued with approximately the same amount of the cholesterol in the food quantity offered, the hyperlipemia decreased gradually, all fractions participating; then, at the 139th day, larger amounts of the cholesterol in ether solution were added to the food offered, and the blood lipids increased with approximately the same lipid fraction distribution as occurred at the beginning.

These experiments disclose that fluctuations may occur in the degree of the hyperlipemia in animals fed cholesterol. The analyses of the blood lipids during this experimentally induced hyperlipemia demonstrated a markedly disproportionate 45-fold increase of the cholesterol fraction as compared with the 9-fold increase of the phospholipids and the 4.5-fold increase of the neutral fat. The nonprotein nitrogen and sugar values of the blood in this form of hyperlipemia remained unchanged during the feeding experiment. A significant feature disclosed by these studies is the ability of the rabbit's metabolism to provide the large content of fatty acids for the neutral fat fraction, the phospholipid fraction, and especially for the cholesterol ester fraction in the increased lipids of the blood. Another item of interest is the relatively constant maintenance of a 70% to 75% ratio of cholesterol ester to the total cholesterol fraction in the blood lipids. The marked differences in the fractional distribution of the lipids of the blood in the hyperlipemias produced in rabbits by supplemental feeding of cream, cream containing 0.5% cholesterol, cream containing 1% cholesterol, and cholesterol without cream are graphically compared in Figure 9.

For the purpose of expressing quantity ratios between solvent and solute in the hyperlipemias of these groups of rabbits, the mEq/liter of fatty acids contained in

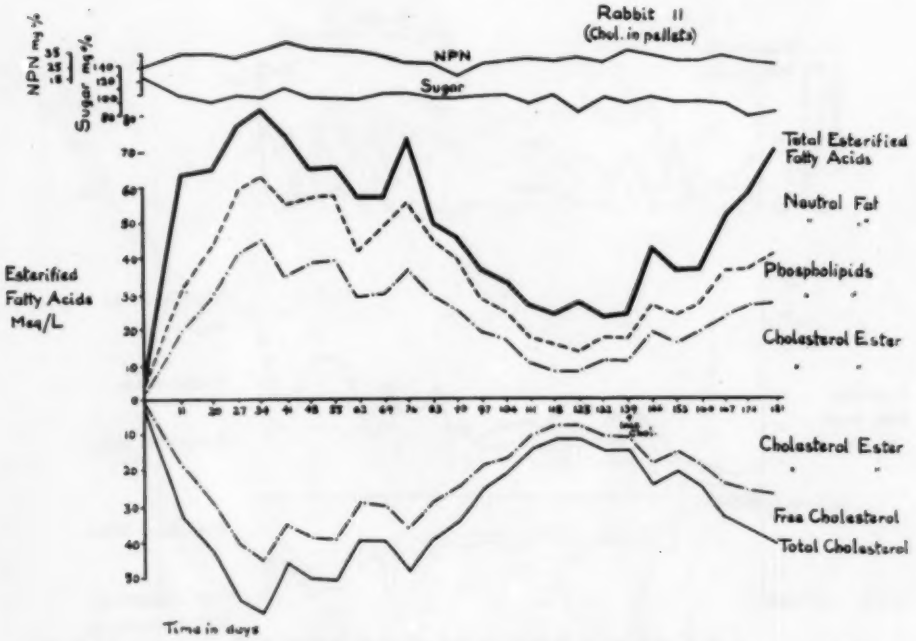

Fig. 7.—Graphs illustrating the composition of the lipids of the blood in the hyperlipemia in rabbits with a diet containing cholesterol without cream.

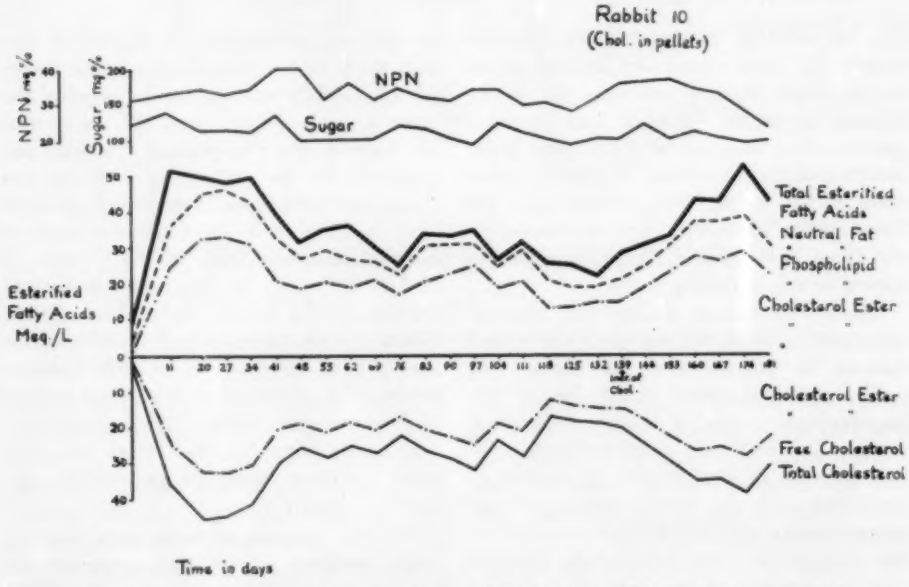

Fig. 8.—Graphs illustrating the composition of the lipids of the blood in the hyperlipemia in rabbits with a diet containing cholesterol without cream.

the neutral fat and phospholipid fractions were placed in ratio with the mEq/liter contained in the acids of the cholesterol ester fraction, to which was added the theoretic amount of mEq/liter of fatty acids that would be bound by the free cholesterol. This correction was simple, because cholesterol esters are consistently about 75% of the total cholesterol. Theoretically, the fatty acids in the blood lipids of the rabbits given cream should be largely of the kinds contained in cream. Table 1 expresses a series of these ratios in the hyperlipemias which developed in rabbits fed cream; Table 2, in rabbits fed cream fortified with 0.5% cholesterol; Table 3, in rabbits fed cream fortified with 1% cholesterol, and, finally, in Table 4, in the hyperlipemia of rabbits fed cholesterol without cream.

These results demonstrate that the chemical characteristics of the lipids and the amount of their increase in the hyperlipemia produced in rabbits by feeding cream differ from those observed in the hyperlipemia produced in rabbits by feeding cream with added amounts of cholesterol (0.5% or 1%) and by feeding cholesterol without the cream solvent. In the cream-fed group the ratio (as expressed in this report) of solvent to solute in the hyperlipemia is maintained at or above the initial base level; in the group fed cream with cholesterol, this ratio shifts and the solvent factor in the ratios drops below 1, and in the third group of rabbits, fed cholesterol without cream, the shift in ratio of solvent to solute drops far below 1:1. The graphs in Figures 1 to 8 inclusive demonstrate further that with this shift to ratios of less than 1:1, the degree of hyperlipemia becomes greater and in the cholesterol-fed group reaches the highest level. Such a degree of hyperlipemia, if produced in rabbits by feeding cream, would cause promptly an acute metabolic death associated with hyperglycemia and marked elevation of the nonprotein nitrogen elements of the blood. The composition of the blood lipids in the hyperlipemia with cholesterol feeding is associated with lipid

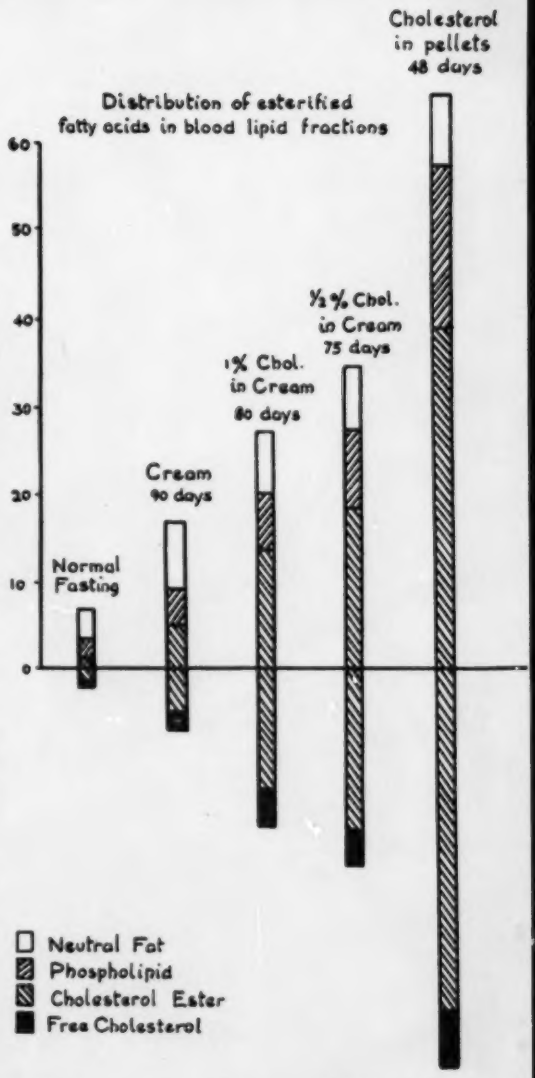

Fig. 9.—The amounts of the total lipids of the blood and of their fractions in the normal rabbit and in the hyperlipemic rabbits given cream, cream with 0.5% cholesterol, cream with 1% cholesterol, and cholesterol without cream are represented in the vertical bars.

saturation of the reticuloendothelial tissues and with deposits in the lining of the aorta and its branches.

The content and fractional distribution of the lipids in the plaques of the aorta of these rabbits were determined. The content and fractional distribution of the lipids in the plaques of the aorta in Rabbits 13 and 15, that had been given cream containing

TABLE 1.—*Analyses of the Blood Lipids in the Hyperlipemia of Rabbits Fed Cream Expressed in Ratios of Solvent (Neutral Fat Plus Phospholipid) to Solute (Total Cholesterol)*

Food	Duration of Feeding, Days	Total Esterified Fatty Acids (Without Free Cholesterol Correction), mEq./L.	Esterified Fatty Acids in Neutral Fat and Phospholipid Fractions, mEq./L.	Esterified Fatty Acids in Cholesterol Ester Fraction with Free Cholesterol Correction, mEq./L.	Ratio
Chow diet with cream					
Rabbit L	0	8	6	3	2:1
	24	20	14.5	7.5	1.8:1
	46	39	31.5	11.5	2.7:1
	80	40	34.0	9	3.7:1
Rabbit F	0	7	5	3	1.6:1
	40	14	10.5	4.5	2.3:1
	80	23	18.5	7.5	2.4:1
	120	24	15.0	12.5	1.2:1
	160	14	11.0	5.0	2.2:1
	180	14	11.5	3.5	3.3:1

TABLE 2.—*Analyses of the Blood Lipids in the Hyperlipemia of Rabbits Fed Cream with 0.5% Cholesterol Expressed in Ratios of Solvent (Neutral Fat Plus Phospholipid) to Solute (Total Cholesterol)*

Food	Duration of Feeding, Days	Total Esterified Fatty Acids (Without Free Cholesterol Correction), mEq./L.	Esterified Fatty Acids in Neutral Fat and Phospholipid Fractions, mEq./L.	Esterified Fatty Acids in Cholesterol Ester Fraction with Free Cholesterol Correction, mEq./L.	Ratio
Chow diet with cream plus 0.5% cholesterol					
Rabbit 13	0	13	11	3	3.6:1
	21	31	17	20	0.85:1
	42	25	14	15	0.93:1
	63	35	22	17	1.3:1
	84	32	13	23	0.6:1
	98	49	21	34	0.6:1
	105	123 acute toxic death			
Rabbit 15	0	9	6	2	3:1
	50	13	8	5	1.6:1
	86	43	22	27	0.81:1
	156	49	28	29	0.96:1

TABLE 3.—*Analyses of the Blood Lipids in the Hyperlipemia of Rabbits Fed Cream with 1% Cholesterol Expressed in Ratios of Solvent (Neutral Fat Plus Phospholipid) to Solute (Total Cholesterol)*

Food	Duration of Feeding, Days	Total Esterified Fatty Acids (Without Free Cholesterol Correction), mEq./L.	Esterified Fatty Acids in Neutral Fat and Phospholipid Fractions, mEq./L.	Esterified Fatty Acids in Cholesterol Ester Fraction with Free Cholesterol Correction, mEq./L.	Ratio
Chow diet with cream plus 1% cholesterol					
Rabbit 5	0	9	8	2	4:1
	28	18	13	9	1.4:1
	63	18	10	11	0.91:1
	119	30	20	25	0.8:1
	140	40	20	26	0.77:1
	144	54*			
Rabbit 2	0	8	7	1.5	4.6:1
	38	28	19	13	1.4:1
	80	27	14	18	0.77:1
	119	24	12	15	0.8:1
	140	31	12	26	0.46:1
	154	34	19	20	0.9:1
	156	157†			

* Acute death; blood sugar level 290 mg. per 100 cc.; NPN, 126 mg. per 100 cc.
† Acute death; blood sugar level 590 mg. per 100 cc.; NPN, 75 mg. per 100 cc.

TABLE 4.—*Analyses of the Blood Lipids in the Hyperlipemia of Rabbits Fed Cholesterol Without Cream Expressed in Ratios of Solvent (Neutral Fat Plus Phospholipid) to Solute (Total Cholesterol)*

Food	Duration of Feeding, Days	Total Esterified Fatty Acids (Without Free Cholesterol Correction), mEq./L.	Esterified Fatty Acids in Neutral Fat and Phospholipid Fractions, mEq./L.	Esterified Fatty Acids in Cholesterol Ester Fraction with Free Cholesterol Correction, mEq./L.	Ratio
Chow diet plus cholesterol					
Rabbit 10	0	8	7.5	2.5	3:1
	20	50	18	43	0.42:1
	34	49	18	42	0.43:1
	62	36	17	26	0.65:1
	90	34	12	29	0.41:1
	125*	25	12	18	0.66:1
	168	42	16	35	0.45:1
Rabbit 11	0	7.5	6.5	3	2.2:1
	34	92	37	60	0.61:1
	62	57	28	40	0.7:1
	90	46	21	35	0.6:1
	118	24	16	12	1.3:1
	125*				
	167	60	28	31	0.9:1

* The amount of cholesterol solution in the pellets was increased.

TABLE 5.—*Total and Fractional Lipid Analyses of Aortic Plaque Compared with Corresponding Analyses of an Average Serum Sample in Rabbit 13, Fed 0.5% Cholesterol in Cream*

	Esterified Fatty Acids		Ratio of Serum (mEq./L.) to Plaque (mEq./Kg.)
	Serum, mEq./L.	Plaques, mEq./Kg.	
Total	48.8	411.4	1:8.43
Phospholipid	11.6	93.4	1:8.05
Cholesterol ester	27.9	160	1:7.2
Neutral fat	9.3	158	1:17
	Cholesterol		
	Serum, Gm./L.	Plaques, Gm./Kg.	
Total	13.4	101.7	1:7.6
Free	2.6	39.8	1:15.3
Ester	10.8	61.9	1:5.73
Ratio, Ester/Total	80.6%	60.8%	

0.5% cholesterol, are given in Tables 5 and 6 and compared in ratios with an average analysis of the lipids of the blood during hyperlipemia of each rabbit. The cholesterol values also are compared. These comparative analyses disclose that the lipid deposits of the plaques in the aorta have a mixed chemical composition similar to the composition of the lipids in the blood, and per tissue weight the plaque lipids are in total, and in the separate fractions, many times more than the concentration of the total lipids and the lipid fractions of the blood. The total cholesterol fraction of the plaques in each rabbit had a comparable increase over that of the serum, and in Rabbit 15 the plaque total cholesterol was increased 16.5-fold.

Similar comparative analyses of the blood lipids in the hyperlipemic rabbits fed a chow diet to which cholesterol had been added

TABLE 6.—*Total and Fractional Lipid Analyses of Aortic Plaque Compared with Corresponding Analyses of an Average Serum Sample in Rabbit 15, Fed 0.5% Cholesterol in Cream*

	Esterified Fatty Acids		Ratio of Serum (mEq./L.) to Plaque (mEq./Kg.)
	Serum, mEq./L.	Plaques, mEq./Kg.	
Total	49	500	1:10.2
Phospholipid	13.8	87	1:6.25
Cholesterol ester	21.3	334	1:15.7
Neutral fat	13.9	79	1:5.7
	Cholesterol		
	Serum, Gm./L.	Plaques, Gm./Kg.	
Total	11.0	181.6	1:16.5
Free	2.7	52.5	1:19.1
Ester	8.25	129.2	1:15.7
Ratio, Ester/Total	75%	71.3%	

and the associated aortic plaques are given in Tables 7 and 8.

These analyses demonstrate that the lipids in the plaques of the aortas of these rabbits are mixtures similar in composition to the lipids of the blood, and in their total and in their fractions are increased many times over the values of the circulating serum lipids. The ratios expressing these increases are much greater in Rabbits 10 and 11, fed cholesterol without cream, than in Rabbits 13 and 15, given 0.5% cholesterol in cream. The comparative ratios for the cholesterol fractions of the serum and the aortic plaques, especially, emphasize the increase in the amounts in the plaques, but with fairly close maintenance of a 70% ratio of ester to total cholesterol. This is not to say that with time, or other factors, such a ratio would persist.

Histological examinations of the livers of the two cholesterol-fed rabbits, Rabbits 10 and 11, demonstrated extensive lipid deposits in the phagocytes, focal infiltrations of large lipophages, and fatty changes of the hepatic cells. The portal systems of Rabbit 10 had focal cellular infiltrations, and the liver of Rabbit 11 had a marked generalized periportal cirrhosis with marked proliferation of the bile ducts. Specific stains demonstrated large deposits of lipids, chiefly intracellular, in the broad fibrous septums that extended between the lobules of liver cells. The lungs and spleen had many large mononuclear phagocytes laden with lipids, and branches of the pulmonary artery had intimal plaques with large lipophages. The medulla of the kidney grossly had pale streaks, which microscopically were foci of lipid deposits with necrosis of the renal tissues. Portions of these deposits had granular debris and also acicular slits where crystalline cholesterol had been present. The glomeruli had fibrous thickened cellular tufts and capsules. The glomerular tufts and their capsules as well as the interstitial cells throughout the kidney had marked lipid deposits. The tubular epithelium had only slight fatty changes. The tissues of those rabbits fed cream with added amounts of cholesterol, and in which the aorta had small plaques, had only slight changes of these tissues.

TABLE 7.—*Total and Fractional Lipid Analyses of Aortic Plaque Compared with Corresponding Analyses of an Average Serum Sample in Rabbit 10, Fed Cholesterol Without Cream*

	Esterified Fatty Acids		Ratio of Serum (mEq./L.) to Plaque (mEq./Kg.)
	Serum, mEq./L.	Plaques, mEq./Kg.	
Total	42.5	667	1:15.7
Phospholipid	8.3	132	1:15.9
Cholesterol ester	22.5	422	1:18.7
Neutral fat	11.7	113	1:9.65
	Cholesterol		
	Serum, Gm./L.	Plaques, Gm./Kg.	
Total	12.1	245	1:20.2
Free	3.39	81.88	1:24.1
Ester	8.71	163.1	1:18.7
Ratio, Ester/Total	71.8%	66.6%	

TABLE 8.—*Total and Fractional Lipid Analyses of Aortic Plaque Compared with Corresponding Analyses of an Average Serum Sample in Rabbit 11, Fed Cholesterol Without Cream*

	Esterified Fatty Acids		Ratio of Serum (mEq./L.) to Plaque (mEq./Kg.)
	Serum, mEq./L.	Plaques, mEq./Kg.	
Total	58.0	803	1:13.8
Phospholipid	11.1	191	1:17.2
Cholesterol ester	26.1	423	1:16.3
Neutral fat	20.8	189	1:9.1
	Cholesterol		
	Serum, Gm./L.	Plaques, Gm./Kg.	
Total	14.3	268	1:18.7
Free	4.2	105	1:25
Ester	10.1	163	1:16.1
Ratio, Ester/Total	70.5%	61%	

COMMENT

Duff,[14] in a summary comment on several reports discussing the relation of arterial wall injury to the evolution of atherosclerosis, stated that there are two elements in the genesis of atherosclerosis: (1) disturbances of lipid metabolism and (2) local tissue factors in arterial walls that permit and promote the deposition of lipids in cer-

tain loci. Fractional chemical analyses of the blood lipids tracing the progress of hyperlipemias experimentally produced in animals, with the purpose of disclosing characteristics of lipid composition significant in the evolution of atherosclerosis, have not been published. Studies of this kind in man are almost impossible, because the requirements of control and of tissue examinations cannot be satisfied. Hence, some of the basic elements of the lipid factor, important in the initial phases of atherosclerosis, must be investigated experimentally in animals.

Rabbits fed cream, as has been described, become hyperlipemic. The composition of the circulating lipids in these animals is such that lipids are not deposited in the lining of the aorta or in the tissues generally during 180 days of an experiment. All fractions of the blood lipids participated in this hyperlipemia, but especially the neutral fat. When the amount of the esterified fatty acids contained in the neutral fat and the phospholipid fractions (solvent) was compared with the amount of fatty acid in the cholesterol ester fraction plus an adjusted amount for the free cholesterol (solutes), the ratio of solvent to solute, as expressed in our report, during the hyperlipemia remained at or above the initial fasting level of the animal. When the lipids of the blood in these cream-fed animals exceeded a critical level, the rabbit had an acute metabolic disorder with marked elevation of the neutral fat fraction of the blood lipids, azotemia, and hyperglycemia, and they died. The absence of lipid deposits in the aorta and tissues generally and the acute metabolic death with its associated chemical changes in the blood contrast the conditions of this hyperlipemia sharply with the chronic wasting disorder associated with the deposition of lipids in the aorta and tissues which occurs with the hyperlipemia of rabbits fed cholesterol. From these observations developed the concept that the lipids of the blood in a hyperlipemia have a solvent component and a solute component. The blood lipids of the cream-fed animal have a large solvent component, while those of the cholesterol-fed animal have a large solute (cholesterol) component. The blood lipids in the hyperlipemia of the latter have the physical properties of particulate material and are taken up by the reticuloendothelial cells, the lipophages of the tissues. Plaques composed of these lipophages appear in the lining of the aorta and arterial branches.

The hyperlipemia developed in rabbits fed cream with 0.5% and 1% added cholesterol is appreciably greater than the hyperlipemia developing in animals fed cream alone. Some of these rabbits also had an acute metabolic disorder with marked elevation of the neutral fat fraction, and several had small lipid deposits in the lining of the aorta but only a minimal number of phagocytes with lipid inclusions in the reticuloendothelial tissues. The ratio of solvent to solute in the blood lipids, as recorded in Tables 2 and 3, was near or slightly below 1 : 1. In contrast with this group, the hyperlipemia of the rabbits fed cholesterol rose to a much higher level than that in all of the others. The amount of cholesterol added to the diet was considered to be moderate rather than excessive. The ratio of solvent to solute in the blood lipids, as expressed in Table 4, dropped in these cholesterol-fed rabbits to much below the 1 : 1 ratio. The lipids of the blood with this composition were deposited in large amounts in the tissue phagocytes, generally, and in plaques in the lining of the aorta. One of these rabbits had a marked cirrhosis of the liver, and the glomeruli of the kidneys in both rabbits had lipid deposits, lipophages, and reactive tissue changes.[18]

TABLE 9.—*Comparison of Lipid Composition of Blood Plasma and Arterial Tissues* *

	Blood Plasma	Intima	Early Plaques	Media
Free cholesterol	14.1	14.2	16.2	17.3
Cholesterol esters	38.3	38.6	38.5	16.7
Phospholipids	22.8	20.1	19.0	34.1
Neutral fat, etc.....	23.3	27.1	26.3	31.9

* The values are given as percentage of total lipid.

Hirsch and Weinhouse, in 1943,[15] presented data on the lipid composition of intimal lesions of the aorta and blood plasma in the human (Table 9). They emphasized the quantitative similarity of the distribution of the lipid fractions in the normal intima, the blood plasma, and the early fatty deposits in the aorta. The total lipid content in the fatty plaques was increased, all fractions participating proportionally.

Tables 5 to 8 contain the results of analyses of the lipids deposited in plaques in the aortas of two rabbits fed 0.5% cholesterol in cream and in the aortas of two rabbits fed cholesterol. These are compared with an average blood lipid analysis made during the hyperlipemia of each animal. The total lipids of the cholesterol-cream-fed rabbits were increased in the plaques 8- to 10-fold over the plasma lipids, and those of the cholesterol-fed rabbits were increased 14- to 16-fold. The separate lipid fractions in the first group were increased in the same range except the neutral fat, which was increased 17-fold in one rabbit but only 5.7-fold in the other. The separate lipid fractions of the plaques in the cholesterol-fed group practically parallel the total lipid increase of the plaques (16- to 19-fold) except the neutral fat. In these two animals the neutral fat increased only about nine-fold. These comparative analyses demonstrate that the aortic plaques are lipid mixtures. They also favor the conclusion that the lipids deposited in the tissues are in approximately the same proportional distribution as they are in the blood plasma lipids, with the exception of the neutral fat (solute) fraction. The smaller proportionate amount of the neutral fat, with the other fractions nearly equal, suggests that a larger proportion of this fraction (solvent) has been removed. If this process occurs, lipid materials deposited originally in the same composition as those of the blood can lose solvent and acquire more and more those properties which stimulate tissue phagocytosis. The process of a concentration of solvent in lipid deposits to a saturation level where cholesterol separates in crystalline form is indicated by the tissue structure of many atheromas in human atherosclerosis and also by the structure of the lipid deposits (streaks) in the interstitial tissues of the kidneys of the rabbits that were fed cholesterol. The lipid deposits in the kidneys had large lipophages and also acicular slits where crystalline cholesterol had been dissolved. Similar acicular slits are observed regularly in atheromas of the human aorta, sometimes in granulation tissues with foreign-body giant cells.

According to Kellner,[16] experiments lend support to the filtration concept of the pathogenesis of atherosclerosis. Proteins and lipids of the serum normally pass across the endothelium into the walls of blood vessels. Where excessive amounts of the larger-size lipid particles infiltrate the wall, the removal of these particles is incomplete and some remain behind to initiate the process of atherosclerosis. Kellner stated further that atherosclerosis thus may result from qualitative and quantitative changes in the serum lipoproteins that filter constantly across the wall of blood vessels or from local structural changes inherent in the vessel wall or resulting from age or disease, that serve to hamper the normal passage of these fatty substances.

Lehninger,[17] in a discussion on the relation of lipids and lipid metabolism to the atherosclerotic problem, stated that in order for lipids as a class to become soluble and mobile the chemical "device" of lipoprotein has been evolved. The use of this "device," however, should not be carried beyond the clear understanding that the lipoproteins, at present, are a lipoprotein complex such as occurs in an emulsion rather than a definite chemical entity.

Hirsch and Weinhouse, in 1943,[15] expressed a view similar to the opinion of Kellner. Lipid complexes infiltrated from the blood plasma into the vessel wall agglomerate into larger particles and stimulate phagocytosis. They recognized two factors in the evolution of atherosclerosis: (1) the

lipid complex and (2) a tissue factor concerned with the deposition of the lipids. They stated that, although cholesteremia may favor the development of atherosclerosis, the lesions in this vascular disorder develop in adult life without an appreciable elevation of the blood cholesterol or obvious abnormality of the blood lipids. This suggests that factors in the tissues of the blood vessels leading to the deposition of the lipids in the intima are important in the causation of the disease. After the lipids are deposited and are in tissues in coarse particulate form, the subsequent stages of atherosclerosis follow.

The present study is an investigation of the lipid factor in the problem of atherosclerosis, undertaken with the purpose of disclosing, if possible, a relation between the chemical composition of experimentally produced hyperlipemias and the evolution of atherosclerosis. It is clear that gradual saturation of the lipids of the blood by cholesterol esters and cholesterol, despite the maintenance of a 70% to 75% ratio in the hyperlipemia of cholesterol-fed rabbits, results in a physical imbalance of the circulating lipids and the formation of a lipid complex that stimulates phagocytosis in arterial and other tissues of the body. This imbalance of the lipid system produced in the animal experimentally is extreme and occurs in a relatively short time. Under other metabolic conditions similar, although small, changes of solvent and solute with time may accomplish, in transport or in deposit, a similar result.

SUMMARY

The lipids of the blood are transported as particles of mixed chemical composition, emulsified in an aqueous medium containing protein and other organic and inorganic solutes. There are two systems in the blood; the one, the emulsified lipid, the other, the aqueous, in which the lipids are suspended. Each system has a solvent in which solutes are dissolved.

For practical purposes the lipids of the blood consist of three main classes: (1) the neutral fats, namely, the triglycerides of the saturated and unsaturated fatty acids; (2) the phosphatides, which are related closely in chemical composition to the neutral fats, and (3) the sterols, cholesterol and its esters.

Esterified fatty acids are integral compounds of all of these lipid fractions except the free cholesterol. The amounts of the fatty acids in the various fractions of the blood lipids are significant data in the evaluation of hyperlipemic disorders.

The emulsified lipids in the blood are like a solvent and its solutes, whose chemical and physical properties accordingly may vary. Lipids liquid at body temperature, such as olein, are solvents for those solid at this temperature. In some hyperlipemias the solvent lipids are in excess; in others the solutes approach the supersaturation level.

The hyperlipemia produced in rabbits by the supplemental feeding of cream is not associated with lipid deposits in the tissues or lining of the aorta. The ratio of solvent to solutes in this hyperlipemia remains at or above the initial fasting level of the blood lipids.

The hyperlipemia produced in rabbits by supplemental feeding of cream containing 0.5% or 1% cholesterol is greater than when cream alone is given. The ratio of solvents to solutes in this hyperlipemia decreases from above 1:1 to this level or below, and in several of these animals atheromas formed in the lining of the aorta but the tissue phagocytes had practically no lipid deposits.

The hyperlipemia produced in rabbits by the supplemental feeding of cholesterol without cream is much greater than that in both of the other groups. Lipids in large amounts are deposited in the tissue phagocytes and in plaques in the lining of the aorta and arteries. The ratio of solvent to solute in the blood lipids of this hyperlipemia is much below a 1:1 ratio. Blood lipids of this composition have a disproportionately high content of cholesterol esters and free cholesterol and have properties similar to those of a par-

ticulate substance. Such lipid complexes stimulate phagocytosis (lipophages).

Analyses of the lipid plaques in the lining of the aortas of the rabbits fed cream and cholesterol and of those in the aortas of rabbits fed cholesterol demonstrate that the plaques are not pure lipids but rather are mixtures, composed of neutral fats, phospholipids, cholesterol, and cholesterol esters. The total lipids in the plaques of the cholesterol-cream-fed rabbits were increased to 8 to 10 times the values of the total lipids (average analysis) in the serum of these animals, and in the cholesterol-fed rabbits, 14 to 16 times. In the first group of rabbits, each lipid fraction in the plaques was increased approximately the same amount over the corresponding serum lipid fraction, except the neutral fat fraction. In one rabbit the neutral fat fraction was increased more than the others, and in the other it was increased much less. The amount of increase of the separate lipid fractions in the plaques of the cholesterol-fed animals over the corresponding serum lipid fractions was practically the same, 16- to 19-fold, except the neutral fat, which was increased only 9-fold.

These comparative analyses demonstrating that the aortic plaques are mixtures of lipids point to the probability that the lipids at first are deposited in the lining of the aorta in approximately the same fractional distribution as they are in the lipids of the plasma. The exception to this could be the neutral fat (solvent) fraction. The smaller increase in the amount of this fraction in comparison with the others, which are increased almost equally in the plaques, suggests that a portion of this fraction (solvent) could have been removed. If this process is in effect, lipids deposited originally in the same composition as those of the blood lose solvent (or add solute) and acquire more and more those properties which stimulate phagocytosis. The acicular slits found in atherosclerotic plaques and other tissues of the human body, clefts where crystalline cholesterol has been dissolved, lend supportive evidence for such a physical change of the lipids in the tissues. Crystals are formed in tissues according to the general principles of crystallization, namely, the supersaturation of a solvent by a crystalloid, through concentration of the solvent or the addition of the crystalline solute.

Experimental atherosclerosis resulting from the saturation of the blood lipids by cholesterol esters and cholesterol takes place in a short time in tissues of an animal presumed to be normal. The blood lipid composition doubtless is dominant under these conditions, but tissue factors are significant in determining the sites of deposition.

The differences in the proportions of the blood lipid fractions and the tissue studies in these experimentally produced hyperlipemias suggest that when tissue lipids approach in composition the saturation of solvent by solute, such as cholesterol esters and cholesterol, whether in transport or in deposit, they become significant causal agents in initiating the evolution of atherosclerotic lesions.

The structural changes of the liver and of the glomeruli in the kidneys of the cholesterol-fed rabbits are tissue effects of the lipids beyond those of simple deposition.

REFERENCES

1. Hilditch, T. P.: Brit. J. Nutrition **3**:347-354, 1949.

2. Bauer, F. C., Jr., and Hirsch, E. F.: Arch. Biochem. **20**:242-250 (Feb.) 1949.

3. Nailor, R.; Bauer, F. C., Jr., and Hirsch, E. F.: Arch. Biochem. **54**:201-205 (Jan.) 1955.

4. Bauer, F. C., Jr., and Hirsch, E. F.: Arch. Biochem. **23**:137-140 (Aug.) 1949.

5. Hirsch, E. F.; Carbonaro, L.; Biggs, A. D., and Phillips, F. L.: A. M. A. Am. J. Dis. Child. **86**:721-725 (Dec.) 1953.

6. Hirsch, E. F., and Carbonaro, L.: Arch. Int. Med. **86**:519-532 (Oct.) 1950.

7. Hirsch, E. F.; Phibbs, B. P., and Carbonaro, L.: A. M. A. Arch. Int. Med. **91**:106-117 (Jan.) 1953.

8. Bauer, F. C., Jr.; Johnson, G. C.; Carbonaro, L., and Hirsch, E. F.: A. M. A. Arch. Path. **51**: 441-445 (April) 1951.

9. Johnson, G. C.; Bauer, F. C., Jr.; Hirsch, E. F., and Carbonaro, L.: A. M. A. Arch. Path. **52**:115-118 (Aug.) 1951.

10. Bauer, F. C., Jr.; Hirsch, E. F.; Carbonaro, L., and Johnson, G. C.: A. M. A. Arch. Path. **56:** 135-141 (Aug.) 1953.

11. Hirsch, E. F., and Nailor, R.: A. M. A. Arch. Path. **59:**419-428 (April) 1955.

12. Weinhouse, S., and Hirsch, E. F.: Arch. Path. **30:**856-867 (Oct.) 1940.

13. Katz, L. N., and Stamler, J.: Experimental Atherosclerosis, Springfield, Ill., Charles C Thomas, Publisher, 1952.

14. Duff, G. L., in Symposium on Atherosclerosis, National Academy of Sciences, National Research Council, Publication 338, 1955, pp. 121-128.

15. Hirsch, E. F., and Weinhouse, S.: Physiol. Rev. **23:**185-202 (July) 1943

16. Kellner, A., in Symposium on Atherosclerosis, National Academy of Sciences, National Research Council, Publication 338, 1955, p. 47.

17. Lehninger, A. L., in Symposium on Atherosclerosis, National Academy of Sciences, National Research Council, Publication 338, 1955, pp. 139-150.

18. Hartroft, W. S.: Am. J. Path. **31:**381-397 (May-June) 1955.

19. Gofman, J. W.; Glazier, F.; Tamplin, A., Stresower, B., and De Lalla, O.: Physiol. Rev. **34:** 589-607 (July) 1954.

The Mechanism of Shock Produced by Means of Staphylococcal Toxin

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The lethal effect of staphylococcal alpha toxin has long been known [1]; yet the precise mechanisms whereby such toxins produce death in animals and man have not been elucidated. Indeed, the mode of action of bacterial toxins in general is obscure. Given intravenously in suitable doses, this substance produces profound and rapidly developing shock which invariably results in the death of the animal. The lethal potency of small amounts of this toxin in man is well demonstrated by the Bundaberg disaster.[2]

In a previous paper [3] it was shown that local infiltration of dilute staphylococcal toxin in the neighborhood of small blood vessels produces prolonged and severe spasm and local areas of ischemia. Furthermore, when given intravenously in rabbits in sublethal doses, it acts selectively on the renal blood vessels, producing marked renal venous spasm and passive congestion of the kidney with subsequent cortical necrosis.[4] These findings suggested that the lethal effect of larger doses of the toxin might similarly result from vascular spasm in a more vital area of the body. In the present study it will be shown that when staphylococcal toxin is given intravenously in lethal amounts to various species of animals, there is a progressive rise in portal vein pressure and sequestration of large amounts of blood in the liver and splanchnic circulation. Furthermore, it will be shown that this phenomenon accompanies *pari passu* the fall in central blood pressure, and is not associated with elevation of caval pressure.

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MATERIALS AND METHODS

Animals.—Adult 8-10 kg. mongrel dogs of both sexes and market-bred 2-3 kg. hybrid albino rabbits were used throughout these experiments. The mice used were stock laboratory mice of the A. strain.

Preparation and Standardization of the Toxin.—The methods of preparation and a standardization of the toxin were described in a previous paper.[3] The dose used with this particular preparation was 1.0 cc. per kilogram of body weight of a 1:10 saline dilution. This was found to be uniformly lethal in dogs and rabbits within 45 minutes.

Tagging[5] of the Red Blood Cells with Cr^{51}.—Several mice were heparinized with 0.1 cc. (1 mg.) of heparin, and five minutes later blood was collected by decapitation. The blood was pooled and heparinized further. An amount of 4 cc. of the pooled blood was tagged by adding 0.2 cc. of a 1:50 dilution of Cr^{51}. This was thoroughly mixed and allowed to stand for 30 minutes at 37 C. The plasma was then taken off and the RBC washed three times in saline and resuspended to the original volume in saline.

Blood-Sugar Determinations.—These were done on fasting animals by the standard Folin-Wu technique.

EXPERIMENTAL FINDINGS

1. General Observations on the Lethal Effect of Staphylococcal Toxin

When the standard lethal dose of staphylococcal toxin is given intravenously in rabbits, a uniformly reproducible result occurs. Within 10 to 30 minutes, in the unanesthetized animal there occurs a very marked

degree of hyperpnea followed by prostration and clonic convulsions of the whole body. The pupils become markedly constricted, and the animal dies rapidly after the onset of convulsions. When this experiment is repeated with the animal under anesthesia with the abdomen open so as to reveal the abdominal viscera, there occurs initially a rather marked cyclical spasm in the smaller branches of the mesenteric veins and arteries. Segmental areas of these veins go into extreme spasm, which lasts but a few minutes and is followed by dilatation. During these periods of cyclical spasm the bowel becomes slightly bluish and shows frequent hyperperistaltic rushes. Arterial pulsation is still evident, though reduced in the face of extreme venous spasm. At this time the liver becomes increasingly engorged and dark bluish-red. Meanwhile, the main portal vein appears distended, and the portal venous pressure appears elevated to the palpating finger. Immediately preceding the terminal episode the veins throughout the mesentery develop an extreme degree of spasm, which persists until death. At autopsy one sees that the most striking finding is the extreme engorgement of the liver. On section large quantities of dark blood exude from the surfaces. There is frequently a similar degree of engorgement of the kidneys. The heart and lungs appear grossly normal, although rarely in the rabbit, but never in the dog, pulmonary edema is found. General anesthesia did not appear to influence the lethal potentiality of the toxin.

These initial observations in the rabbit suggested the possibility of sequestration of large volumes of blood in the liver, producing a diminution in venous return to the right side of the heart with resulting shock and death. Since a similar picture might conceivably be produced by acute right-sided heart failure, it was believed necessary to compare simultaneously pressures in the portal and caval systems. Accordingly, this work was repeated in dogs.

In dogs, the lethal dose was determined and found to agree closely with that of the rabbit on a weight basis. When given intravenously in unanesthetized dogs, the toxin initially produced a considerable degree of hyperpnea lasting about two minutes, followed by marked evidence of hyperperistalsis and defecation. The majority of animals then became prostrate and died rapidly in a shock-like state. At autopsy the findings were similar to those in the rabbit. Again, the liver was enormously engorged, and the heart and lungs appeared normal, though the kidneys were uniformly enlarged and congested; the spleen, on the other hand, was invariably contracted. There was no evidence of dilatation of the right side of the heart.

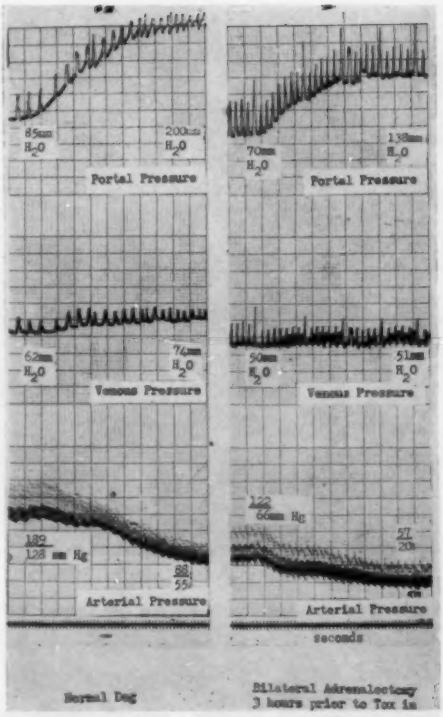
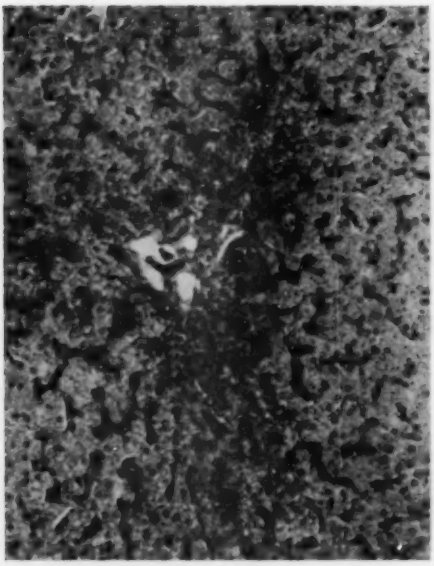
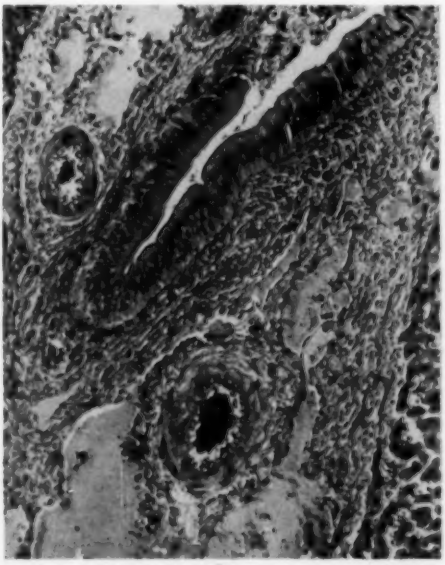


Fig. 1.—*A*, the immediate effect of a lethal dose of Staphylococcal toxin on portal, caval, and systemic pressures. The elevation in portal pressure is unaccompanied by significant change in inferior vena caval pressure. *B*, the same experiment after bilateral adrenalectomy. The response is essentially similar in both instances.

Elevation of the Portal Pressure. — In order to study the pressure relationships



A



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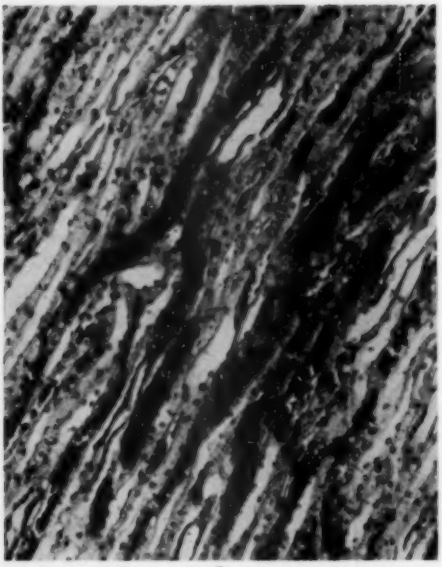

C

Fig. 2.—*A*, extreme congestion of hepatic sinusoid vessels in the rabbit. This was more marked in the central area. *B*, prominent dilatation of periportal lymphatics in the liver of a dog dying 45 minutes after a lethal dose of staphylococcal toxin. *C*, engorgement in the vera recta in the renal medulla of the kidney of a dog dying 45 minutes after a lethal dose of staphylococcal toxin.

within the portal and caval systems more precisely, a cardiac catheter was introduced by way of a branch of the splenic vein into the main portal vein, and another into the vena cava through the femoral vein. Arterial blood pressure was taken by cannulation of the femoral artery. The animal was then heparinized, using 1.0 mg. per kilogram of body weight; then the abdomen was surgically closed after threading the catheter into the main portal vein. Control readings of the three pressures and an electrocardiogram were recorded on a Sanborn Polyvisograph. The midchest level was taken as the zero point. Pressure measurements were taken with the abdomen closed. It will be seen from the graph reproduced in Figure 1*A* that following injection of the full lethal dose of toxin, there occurred an immediate rise in portal pressure accompanied by a fall in systemic blood pressure. The rise in portal venous pressure occurred concurrently with the injection of toxin and lasted from 6 to 10 minutes. Thereafter, the portal pressure returned to control levels, though the engorgement of the liver became progressively greater. Caval venous pressure remained at control levels or actually fell below

control levels during the subsequent course of the experiment, these findings showing that the rise in portal pressure was not a result of right heart failure. The rise in portal pressure was invariably accompanied by marked hepatic engorgement.

Histologic Findings in the Engorged Liver.—Stained sections of the liver, removed soon after death, consistently showed engorgement of the sinusoidal capillaries and central venules (Fig. 2*A*), indicating that the increase in volume and weight of the liver was the result of pooling of blood in the sinusoidal capillaries. The periportal lymphatics also showed considerable distention (Fig. 2*B*).

Prevention of the Rise in Portal Pressure by the Construction of an Eck Fistula.—If the accumulation of blood in the hepatic and portal circuits was prevented by providing an alternate route to the right heart, an amelioration of the lethal effects of the toxin might be expected. To test this hypothesis, Eck fistulas were made in three dogs one week prior to the experiment. By this time the animals had recovered from the surgery and were in a vigorous state of health. They were anesthetized and cannulated in the standard manner. The results of a lethal injection of toxin are shown in Figure 3. It will be seen that there is no rise in the portal pressure; moreover, the animals tolerated the single injection of toxin without the rapid and progressive fall in blood pressure which occurred in the control group. Yet, on increasing the amount of toxin, death occurred. It was of interest to find severe hepatic engorgement in all these animals in spite of the complete diversion of portal blood flow. This finding suggests that the circulating blood volume was effectively, though less rapidly, reduced by the inflow of blood into the liver from the hepatic artery.

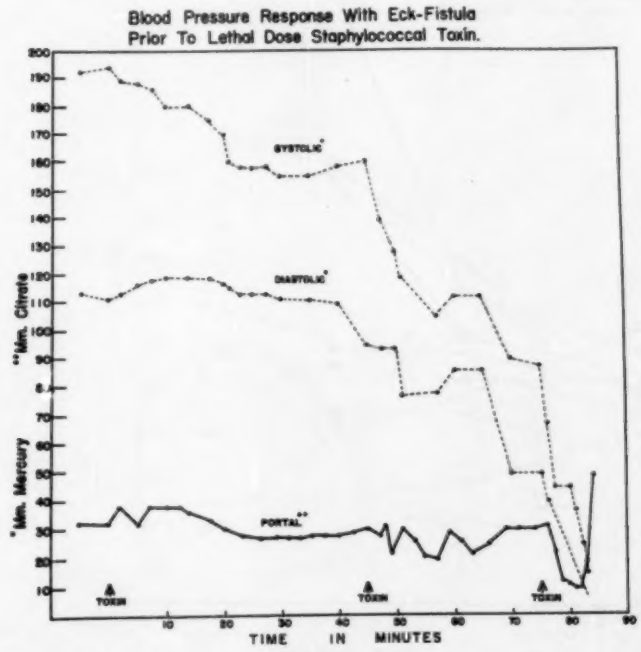

Fig. 3.—Systemic and portal vein pressures of a dog in which an end-to-side Eck fistula had been made one week prior to toxin injection. The initial standard dose of toxin was unassociated with marked change in systemic or portal pressure. However, repeated doses of toxin invariably produced progressive hypotension and death.

Bilateral Adrenalectomy.—As shown by Bradley and others,[6] epinephrine release is associated with both systemic and portal hypertension. In order to determine whether the portal hypertension seen in animals given

a lethal dose of staphylococcal toxin was merely the result of epinephrine release, the previous experiments were repeated on acutely adrenalectomized animals. As shown in Figure 1*B*, the early rise in portal pressure was consistently found in the adrenalectomized animal.

Effect of Hepatectomy on the Lethal Effect of the Toxin.—To obviate the sequestration of blood in the liver, hepatectomy was performed in three Eck-fistula dogs immediately prior to the injection of the toxin. These hepatectomies were rapidly accomplished with minimal blood loss by temporarily clamping the aorta in the chest during the operation. Any blood loss was immediately replaced, and at the conclusion of the operation the animals were in good condition. The tolerance of these animals to toxin was then tested. All animals in this group died within 30 minutes to the standard dose of toxin. No protective effect of hepatectomy was apparent; indeed, these animals appeared less resistant to the toxin than were the controls.

Blood-Glucose and Leucocyte Response to the Toxin.—It has been shown by many observers that Gram-negative endotoxins produce a transient leucopenia and hyperglycemia.* Similarly, in the present experiments the intravenous administration of staphylococcal toxin was regularly followed by hyperglycemia and leucopenia within 30 to 90 minutes (Figs. 4 and 5). Adrenalectomized animals and animals with Eck

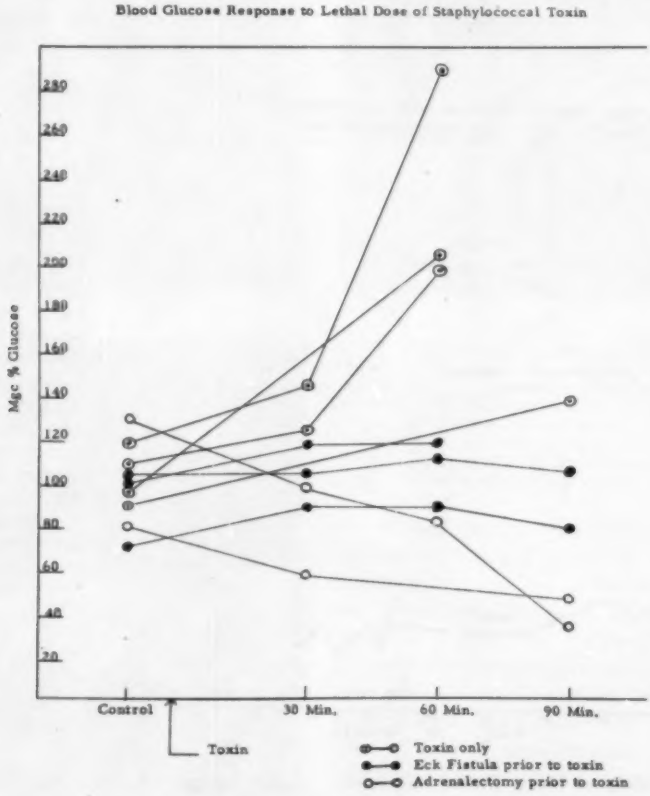

Figure 4

* References 7 and 8.

fistulas consistently failed to show the hyperglycemic response. In the case of the adrenalectomized animals these findings might suggest that the hyperglycemia was a reflection of epinephrine release. The reason for the failure of the response in the case of the animal with Eck fistulas is not readily apparent. On the other hand, all animals regularly showed the leucopenic effect. Microscopic sections of the lungs regularly demonstrated aggregation of leucocytes along the endothelium of the small pulmonary vessels.

Leukocyte Response to Lethal Dose of Staphylococcal Toxin

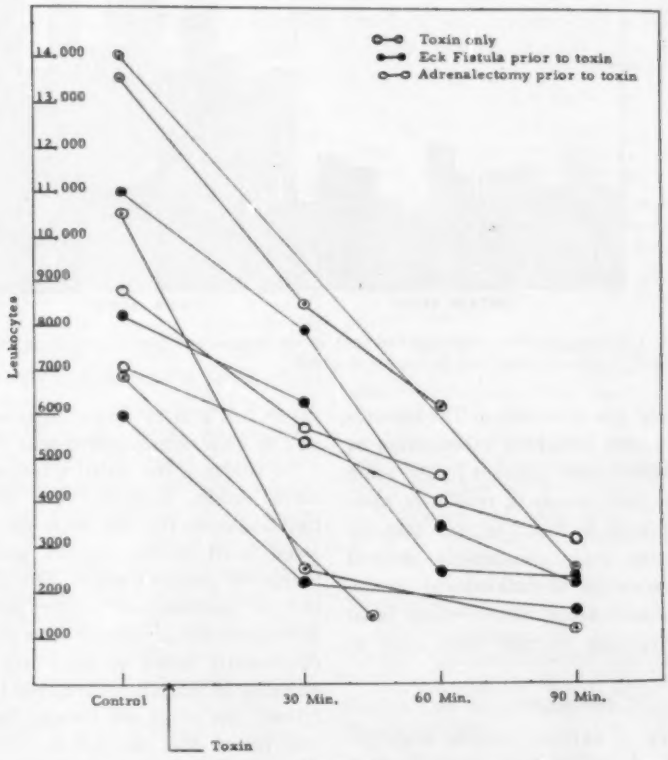

Figure 5

2. Sequestration of Red Blood Cells in the Hepatic and Portal Area

The consistent findings of a significant increase in the mass of the liver suggested that blood was being sequestered in this area. To study this phenomenon further 24 mice were given 0.2 cc. of Cr^{51}-tagged red blood cells by intravenous injection through a tail vein. After the cells were allowed to disperse throughout the body for 10 minutes, 12 of the mice were given a lethal dose of staphylococcal toxin. All 12 died within 45 minutes. Immediately after death the whole mouse was placed in a 50 cc. Lusteroid tube and counted in the Texas well counter. Then the liver and gastrointestinal tract were removed together, care being taken to ligate the main vessels so that the blood was contained in the hepatic and portal system. In this manner the radioactivity could be rapidly assayed and the ratio of Cr^{51} present in the hepatic and portal systems to that in

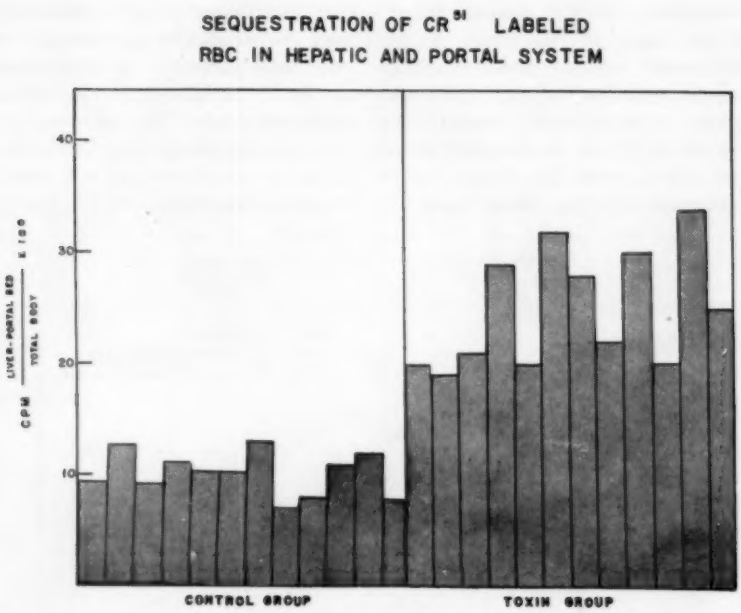

Fig. 6.—The aggregation of labeled red cells in the hepatic and portal systems in mice dying as a result of toxin injection; and in a control group.

the whole body was determined. The remaining 12 mice were killed by etherization at the same intervals and counted in the same manner. The comparison of results is summarized in Figure 6. It will be seen that the group receiving toxin consistently showed a greater percentage of radioactivity in the portal and hepatic areas, these findings indicating sequestration of red blood cells in this area.

COMMENT

The finding of extreme hepatic engorgement in animals dying with staphylococcal toxemia is not peculiar to this particular toxin. Similar engorgement of the liver has also been described in anaphylactic, histamine, and peptone shock,† and is commonly seen in animals dying with overwhelming bacterial infection within the peritoneal cavity. It can be produced not only by the experimental administration of the staphylococcal alpha toxin, as shown in the present paper, but also by Gram-negative endotoxin and by Clostridium perfringens alpha toxin.‡

In studying the lethal effect of staphylococcal toxin, Kellaway and co-workers [11] have indicated that the toxin produces right-sided heart failure due to arterial spasm within the pulmonary tree. This finding could not be corroborated in the present work. Measurements of inferior vena cava pressure consistently failed to show any significant elevation as an indication of right-sided heart failure. All in all, the present findings indicate rather that the fall in systemic blood pressure is a result of diminished venous return to the right side of the heart. In the doses of toxin administered, no conduction disturbances were revealed by electrocardiogram, the heart-beat continued to be forceful and regular though rapid for considerable periods after the injection of the toxin, and direct visualization of the heart immediately prior to death showed small undilated chambers. These findings indicate

† References 9 and 10.

‡ Thal, A. P.: Unpublished observations, 1954.

that the action of the toxin is probably peripheral to the heart and to the vessels of the lung.

Previous work on the effect of sublethal doses of staphylococcal toxin indicates that the substance produces a selective spasm in the blood vessels of the kidney, with considerable renal engorgement and renal venous spasm.[4] Accordingly, it was believed that the extreme hepatic engorgement produced by lethal doses of the toxin might be a reflection of selective spasm within the hepatic venous system. Histologic studies consistently showed extreme dilatation of the sinusoidal vessels of the liver and engorgement of the central veins. Whether or not this capillary engorgement is a direct vasoparalytic effect of the toxin or is the result of spasm on the venous side of the capillary bed is not yet clear. This finding would suggest that the site of such spasm, if it exists, must lie between the central veins and the larger muscular-walled hepatic veins. On the other hand, similar turgidity of the liver might manifestly be produced simply by loss of capillary tone and passive dilatation of the capillary system in a sponge-like manner. The hepatic engorgement might then be but a local manifestation of generalized relaxation of capillary tone induced by the toxin. This concept is also supported in part by the finding that other organs apart from the liver show capillary engorgement and dilatation. Medullary engorgement of the kidney in response to lethal and sublethal doses of the toxin is a consistent finding (Fig. 2*C*) and, incidentally, is often described in fatal cases of shock in the human.

By providing an alternate route to the heart in animals with Eck fistulae, a definite ameliorating effect on the lethal manifestations of the toxin was noted. Yet these animals ultimately succumbed to a repeated dose of toxin and at autopsy showed engorgement of the liver which, though of a slighter degree than that usually seen, was nevertheless obvious. The afferent blood flow to the liver in these animals came solely from the hepatic artery. To eliminate further this question of sequestration of blood in the liver, hepatectomized animals were studied. While this, of necessity, brings about a more complex state of affairs by removing an important site of detoxification, yet the absence of any protective effect further serves to emphasize the possibility that the sequestration of blood within the capillaries of the liver was merely part of a generalized capillary pooling throughout the body.

REFERENCES

1. Neisser, M., and Levaditi, C.: Compt. rend. Congres. internationale de Méd., Sect. path. gén. et path. expér., Paris, 1900-1901.

2. The Australian Royal Commission of Enquiry into the Fatalities at Bundaberg: Report 1928, Parliamentary Debates **119**:6143-6144, 1928.

3. Thal, A. P., and Egner, W.: Local Effect of Staphylococcal Toxin, A. M. A. Arch. Path. **57**: 392-404, 1954.

4. Thal, A. P.: Selective Renal Vasospasm and Ischemic Renal Necrosis Produced Experimentally with Staphylococcal Toxin, Am. J. Path. **31**:233-259, 1955.

5. Read, R. C.: Studies of Red-Cell Volume and Turnover Using Radiochromium, New England J. Med. **250**:1021-1027, 1954.

6. Bradley, S. E.; Inglefinger, F. J., and Bradley, G. P.: Determinates of Hepatic Haemodynamic, in Visceral Circulation: A Ciba Foundation Symposium, edited by G. E. W. Wolstenholme and others, Boston, Little Brown & Company, 1953.

7. Van Heyningen, W. E.: Bacterial Toxins, Oxford, Blackwell Scientific Publications, 1950.

8. Biedl, A., and Kraus, R.: Experimentelle Studien über Anaphylaxie, Wien. klin. Wchnschr. **22**:363-370, 1909.

9. Mautner, H., and Pick, E. P.: Über die durch Schockgifte erzeugten Zirkulationsstörungen, München. med. Wchnschr. **62**:1141-1143, 1915.

10. Dale, H. H., and Laidlaw, P. V.: Histamine Shock, J. Physiol. **52**:355-390, 1919.

11. Kellaway, C. H.; Burnet, F. M., and Williams, F. E.: Pharmacological Action of the Exotoxin of Staphylococcus Aureus, J. Path. & Bact. **33**:889-912, 1930.

Hemosiderin Deposition in Early Atherosclerotic Plaques

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The "vascularization theory" attempts to explain all the changes in atherosclerosis on recurrent hemorrhage into the intima of arteries. The lipid deposits, which are such a characteristic feature of the disease, are thought to be derived from the lipids of the extravasated blood, whether they are at normal or at elevated levels. The fibroplasia is explained by the organization of the clot. The actual leakage of blood is due to the rupture of capillaries which arise mainly from the arterial lumen. Two major objections to this theory have been raised: (1) intimal hemorrhages are thought to be relatively infrequent and have been described only in the end-stages of the disease, and (2) the intima of normal arteries, or of slightly atherosclerotic arteries, is said to have no capillary blood supply. For these reasons, vascularization of the intima with hemorrhage seems to be regarded as the result, not the cause, of the atherosclerotic process.

Indirect evidence has now been obtained which suggests that hemorrhages into atherosclerotic plaques are in fact extraordinarily frequent and that they occur in the earliest as well as in the later stages of the disease. The evidence has been obtained by the demonstration, not of hemorrhage proper, but of one of the sequelae of hemorrhage, hemosiderin formation; and this has been achieved by subjecting gross segments of unfixed arteries to the Prussian-blue reaction. Deposits of hemosiderin, so small that they would be easily missed in thin microscopic sections, become quite evident when stained in this way.

MATERIAL AND METHOD

The material was obtained from approximately 150 autopsies on persons ranging in age from 3 years to 90 years. The majority of the patients were middle-aged or elderly men; a few were in the third or fourth decades, and an occasional child and a few female patients were included in the series. Most of the patients had succumbed to acute or chronic diseases, but in 10 instances death had been sudden and violent and had occurred in presumably normal persons. Autopsy in most of the cases was carried out a few hours after death, but occasionally from one to three days had elapsed.

In each case a 6-in. segment of the thoracic aorta, extending proximally from the celiac axis, was removed, opened anteriorly with scissors (sometimes of the iron-free type), stripped manually of its adventitia and outer media to remove those tissues contaminated by metallic instruments, and rinsed quickly but thoroughly in distilled water. The specimen was then exposed immediately, in an unfixed state, to a freshly prepared solution of equal parts of 10% potassium ferrocyanide (preferably ferricyanide-free) and 20% hydrochloric acid. During these maneuvers, care was taken to avoid contaminating the specimen, or the solutions, with metallic objects. The specimen was left in the reagent at room temperature for half an hour, rinsed quickly in distilled water, and then examined with the naked eye or with a magnifying lens. A photograph of the specimen

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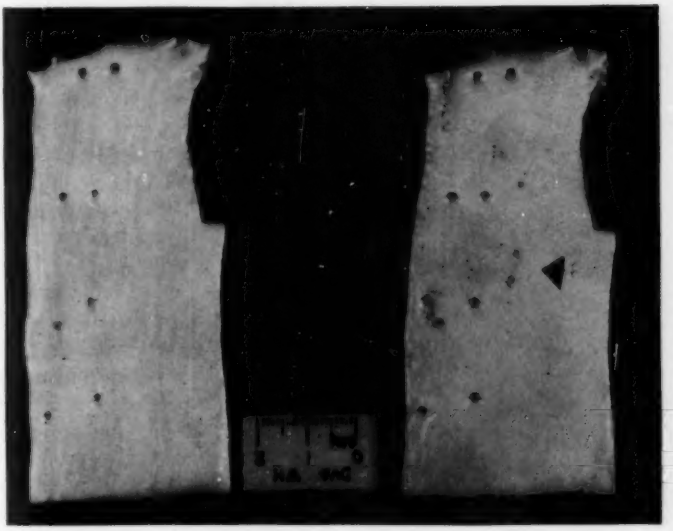
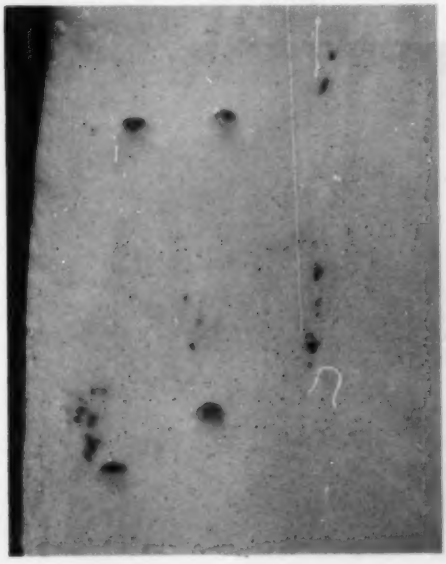


Fig. 1.—Segment of the lower thoracic aorta from a 31-year-old man, reproduced from a colored photograph (Ektachrome) using a Wratten 25 filter. Left, before exposure to Prussian-blue reaction; right, after exposure. Fatty streaks can be seen on the upper half of the photograph on the left, while on the right the arrow points to pinpoint areas of Prussian-blue material in some of the fatty streaks. These same areas are shown in the enlarged photograph in Figure 2.

in color was taken before exposure to the reagents, and immediately afterward.

Procedures similar to the above have been carried out on large segments of the coronary and femoral arteries.

Fig. 2.—Enlargement of part of the photograph of the lower thoracic aorta from a 31-year-old man illustrated in Figure 1. The focal distribution of the Prussian-blue material in only some parts of the fatty streaks is shown.



When iron-staining deposits were present, thick frozen sections of fixed and unfixed material were cut from representative areas. These sections were examined microscopically either in an unstained state or after light counterstaining procedures. Other segments of iron-containing plaques were fixed in formalin, embedded in paraffin, sectioned, and stained by orthodox methods.

OBSERVATIONS

Deposits of Prussian-blue material have been encountered in one or more plaques in the great majority of the cases. The only exceptions were young children, a 14-year-old boy, three young men in the third decade, a 49-year-old man, and two middle-aged women. The youngest to give a positive reaction in the series was 19 years old. The Prussian-blue material was found exclusively in atherosclerotic lesions. When no reaction was obtained, it usually, but not always, meant that no atherosclerotic plaques were present. A marked variation was noted in the amount of stainable material in different cases. Occasionally, only one or two plaques were involved; sometimes, almost every plaque gave the reaction, but as a rule only about half of the plaques showed evidence of discoloration. Points of discoloration of pinpoint size have been noted in fatty streaks

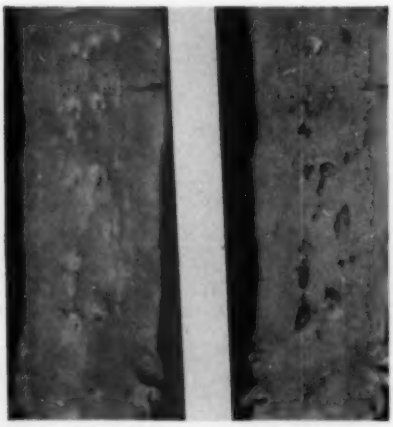


Fig. 3.—Segment of the lower thoracic aorta, reproduced from a colored photograph (Ektachrome) using a Wratten 25 filter. Left, before exposure to the Prussian-blue reaction; right, after exposure. Most of the small pearly plaques and the fatty streaks seen in the unstained specimen show (on the right) appreciable amounts of Prussian-blue material.

in several young men (Figs. 1 and 2), but often these incipient lesions remained unstained. The reaction was most striking in the "pearly" plaques of endarteritis (Fig. 3). Indeed, the deposits have been more impressive in these early lesions than in end-stage plaques. At all stages the deposits were located not infrequently about the periphery of plaques (at points where intimal capillaries are known to be most numerous [1]) (Fig. 4). Surface deposits of blood, or blood lying within capillaries in the superficial tissues, did not give the reaction, even when advanced postmortem degeneration of tissues was present. The reaction was found in plaques with intact intimal surfaces, although it was, of course, also present at the edges of erosions.

The discolorations were just as common in cases of sudden death as in patients who succumbed to long-standing wasting illnesses. They were numerous in the plaques of persons dying suddenly of violent causes. Delay in carrying out the autopsy did not affect the incidence of positive reactions. And in this regard, it was noted that blood which had been stored for several days in the icebox remained negative to the Prussian-blue reaction.

The reaction of atherosclerotic plaques in the coronary and femoral arteries was just as striking as that of plaques in the thoracic aorta.

Microscopically, the best results were obtained from the study of thick frozen sections. The discolorations were found most frequently in the superficial layers of the intima (Fig. 5); less often they were more deeply

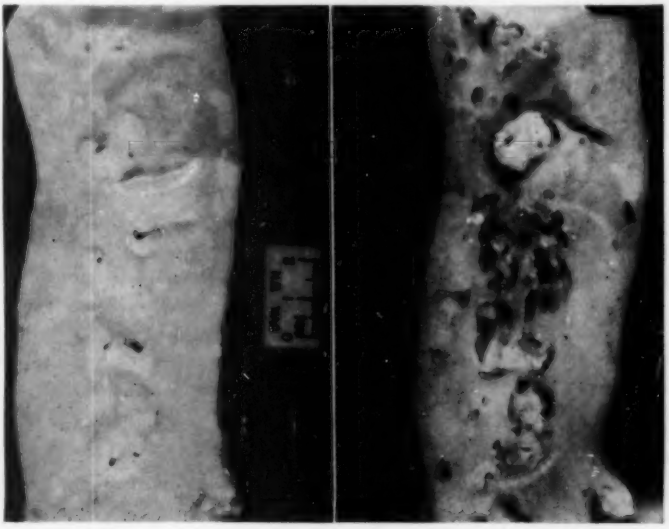


Fig. 4.—Segment of lower thoracic aorta, reproduced from a colored photograph (Ektachrome) using a Wratten 25 filter. Left, before exposure to Prussian-blue reaction; right, after exposure. The specimen shows many atherosclerotic plaques of moderate size, and most of these contain Prussian-blue material, particularly at their periphery.

Fig. 5.—Photomicrograph, using a Wratten 25 filter, of a thick frozen section through an atherosclerotic plaque, exposed previously to the Prussian-blue reaction and counterstained lightly with carbol-fuchsin. A deposit of Prussian-blue material is seen close to the endothelium, and another in the deeper tissues at the edge of an atheroma. Reduced ⅓ from mag. × 50.

situated at the edges of atheromatous foci (Fig. 6), but they were almost never seen in the contents of atheromatous "abscesses." Occasionally they have been found in the outer parts of plaques, close to the muscle layer (Fig. 7). The superficial deposits were finely granular and were usually contained in macrophages; the deeper ones were bulkier and sometimes lay free in the tissues.

By the multiple section technique, capillaries which contained red blood cells could

Fig. 6.—Photomicrograph taken under similar conditions to that illustrated in Figure 5. The Prussian-blue material lies in the deeper parts of the intima at the edge of an atheroma. Reduced about ⅓ from mag. × 50.

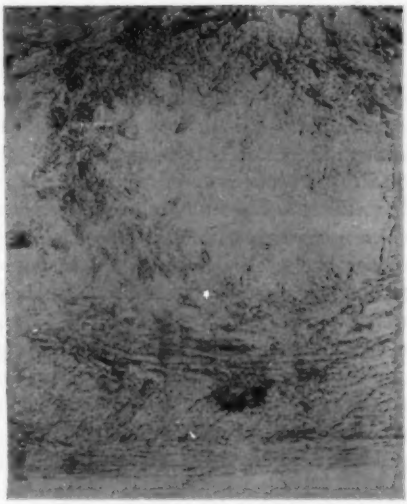


Fig. 7.—Photomicrograph taken under similar conditions to that illustrated in Figure 5. A deposit of Prussian-blue material is shown close to the bottom of the photomicrograph; it lies external to a large atheromatous "abscess," near the medial coat. The adventitia is at the bottom of the figure. Reduced about ⅓ from mag. × 50.

usually be demonstrated in the vicinity of the Prussian-blue material in the advanced lesions (Fig. 8). Capillaries, recently extravasated blood, and Prussian-blue material have

Fig. 8.—Photomicrograph of an unstained, thick frozen section of an atherosclerotic plaque, using a Wratten 25 filter. The plaque had been exposed previously to the Prussian-blue reaction. The lumen of the artery is at the top of the figure, and close to it on the right is a capillary. Significant amounts of Prussian-blue material are seen close to this capillary, notably within the macrophage at left center. Reduced about ⅓ from mag. × 300.

all been seen in the same general location in one plaque (Fig. 9).

More difficulty has been experienced in demonstrating capillaries in earlier lesions, e. g., in fatty streaks and pearly plaques, although these also have been shown to contain Prussian-blue material. Orthodox staining methods have usually failed to show any evidence of vascularization in these incipient lesions; but when the alkaline phosphatase reaction [2] was used on serial sections, structures which we interpret as capillaries could be demonstrated. This latter finding suggests that vascularization of the intima is an early rather than a late manifestation of the disease. It will be discussed in detail in a later report.

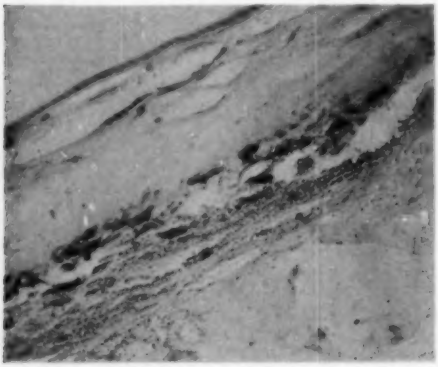


Fig. 9.—Photomicrograph, using a Wratten 25 filter, of a frozen section through an atherosclerotic plaque which contained obvious recent hemorrhage. The plaque had been exposed previously to the Prussian-blue reaction, and the section stained with carbol-fuchsin. Both recently extravasated blood and Prussian-blue material are seen in the same general location, and capillaries could be demonstrated in the vicinity. Reduced about ⅓ from mag. × 50.

COMMENT

The Prussian-blue material noted in this study undoubtedly represents iron. Since it occurs so frequently in atherosclerotic plaques, in early lesions as well as late ones, its possible significance in the pathogenesis of the disease becomes a matter of some interest.

By its reaction the material is ferric iron, and it can therefore be considered to be either ferritin or hemosiderin. Unlike Bunting,[3] when ferricyanide (ferrocyanide-free) is substituted for the ferrocyanide, we have been unable to detect any ferrous component in the deposits. We therefore cannot agree with Bunting that the material "differs from that derived from hemoglobin destruction . . . , which is usually intracellular in location and uniformly ferric." And in this regard, our microscopic studies showed the great mass of the stainable material to be finely granular and to lie within macrophages, often within cells interpreted as foam cells.

It is doubtful also that the material is ferritin, since we have exposed a sample of crystalline ferritin, with a definite iron content, to the Prussian-blue reaction with negative results. And contrary to the suggestions of Gillman and co-workers,[4] we have found no evidence of a generalized cytosiderosis in our cases: as will be described later, there was no relationship between the amount of iron in the aorta and that in other common sites of iron storage.

From the evidence, then, the Prussian-blue material described in this paper is hemosiderin and is probably derived from hemoglobin destruction. Hemosiderin deposits in atherosclerotic plaques probably have little influence per se on the progression of the disease, but the mechanism of their formation may be of great theoretical importance. At least three possibilities deserve consideration.

By the "imbibition theory," the foam cells that accumulate in the intima because of a derangement in the serum lipids are said to possess phagocytic properties.[5] Foam cells in preexistent plaques might therefore take up free hemoglobin from the blood serum and convert it to hemosiderin.[6] By this mechanism, hemosiderin deposition in atherosclerotic plaques would be a purely secondary phenomenon, and of no real significance in the pathogenesis of the disease. However, if the hemosiderin deposits were due simply to phagocytosis of circulating hemoglobin, a similarity should be apparent between the amount deposited in the aorta and that in other organs which are known to store iron

Amount of Haemosiderin in Aorta \ Amount of Haemosiderin in Liver	Marked	Moderate	Slight	None
Marked	0	0	2	5
Moderate	0	0	8	12
Slight	0	0	3	12
None	1	0	3	6

Chart 1.—Comparison of the amount of hemosiderin in the aorta (determined macroscopically) with the amount in the liver (determined microscopically) in each of 50 fatalities.

for future use, notably the liver and spleen. We have failed so far to demonstrate any such similarity. As a routine procedure we have exposed segments of liver and spleen to the Prussian-blue reaction simultaneously with the aorta from the same case. The response of the three tissues, when examined by gross and microscopic methods, has often been quite dissimilar. Striking reactions in the liver and spleen have been associated with minimal reactions in the aorta, and vice versa (see Charts 1 and 2). One case in point might be mentioned: a 49-year-old man who suffered from hemolytic anemia and who had received approximately 50 blood transfusions during the last two years of life. At autopsy, the liver and spleen were loaded with iron, but none was found in fatty streaks of the aorta. Furthermore, it has been noted repeatedly in this study that only some plaques, and only some portions of plaques, showed the reaction. Assuming that all plaques, certainly all early ones, have foam cells in their substance, it seems odd that a few foam cells should have the property of ingesting hemoglobin and converting it to hemosiderin, whereas others have not. The minute deposits of hemosiderin found in the fatty streaks of the aorta of a 31-year-old man, and illustrated in Figure 2, are so focal in distribution that it is difficult to conceive that they resulted from the phagocytosis of iron circulating in the blood stream. On the whole, then, it would appear highly improbable that deposits of ferric iron in atherosclerotic plaques are due simply to phagocytosis of hemoglobin, or other forms of iron in the circulating blood, by preexistent fat-laden macrophages.

Second, an explanation for the presence of hemosiderin in atherosclerotic plaques can be given by the "encrustation theory": Proponents of this theory will claim that it is due to the disintegration of red cells that are enmeshed in tiny mural thrombi. This explanation is a reasonable one for superficial hemosiderin deposits, but not for those that are deep-seated. Occasionally the deposits have been found external to atheromatous foci close to the media, positions which are difficult to explain by migration of the constituents of a thrombus (Fig. 7). Furthermore, it would appear that enmeshed red cells have never been demonstrated in these tiny fibrin thrombi. If they were present, the thrombi would be red in color and, therefore, visible to the naked eye. It is a matter of record that they are not visible. And without

Amount of Haemosiderin in Aorta \ Amount of Haemosiderin in Spleen	Marked	Moderate	Slight	None
Marked	0	0	3	4
Moderate	0	5	7	7
Slight	0	3	6	6
None	1	1	5	3

Chart 2.—Comparison of the amount of hemosiderin in the aorta (determined macroscopically) with the amount in the spleen (determined microscopically) in each of 51 fatalities.

a red cell component, it is difficult to explain either hemosiderin formation or lipid deposition.

Finally, the most reasonable explanation of all is that the ferric iron in atherosclerotic plaques results from the leakage of blood into the intima—from capillary rupture and intimal hemorrhage. The focal nature of hemosiderin deposition in fatty streaks (as in Fig. 2) and their circumferential distribution in older plaques (at points where capillaries are known to be most numerous) support the contention that capillary rupture has occurred in these locations. Additional evidence in this regard is given by our ability to demonstrate definite capillaries in older plaques, and structures interpreted as capillaries in earlier lesions, in close relation to the hemosiderin. There is of course no proof that this was the mechanism at fault: All that can be said is that capillaries show "guilt by association." Further data are needed before this mechanism can be accepted, but, if true, it means that intimal hemorrhages are extraordinarily frequent in the earlier stages of the disease. And if iron can be laid down at points of hemorrhage, it is reasonable to assume that the lipid which is present in normal blood, or in hyperlipemic blood, may be deposited at the same time. At all events, any theory on the pathogenesis of human atherosclerosis must be competent to explain the presence in early and late lesions, not only of lipid and fibrous tissue but of iron as well.

SUMMARY AND CONCLUSIONS

Exposure of gross segments of the unfixed human aorta to the Prussian-blue reaction has revealed numerous deposits of iron lying exclusively within atherosclerotic plaques. The deposits, which are considered to be hemosiderin, have been found in the earliest lesions of the disease as well as in end-stage plaques. Microscopically, the deposits have been found in close proximity to capillaries of the intimal variety. The focal nature of the deposits and their close association with capillaries suggest to us that they mark the spots where intimal hemorrhages have occurred fairly recently. This impression is strengthened by the results of our attempts to exclude other mechanisms by which hemosiderin might have been deposited in these locations.

Prof. John H. Fisher, University of Western Ontario; Prof. John Hamilton, University of Toronto, and other pathologists assisted in obtaining autopsy material for these studies. The photographs were taken by Mr. Don Pulham, of Westminster Hospital.

REFERENCES

1. Winternitz, M. C.; Thomas, R. M., and Le Compte, P.: Biology of Arteriosclerosis, Springfield, Ill., Charles C Thomas, Publisher, 1938.

2. Gomori, G., in Techniques of Histo- and Cytochemistry, edited by David Glick, New York, Interscience Publishers, Inc., 1949.

3. Bunting, H.: Histochemical Analysis of Pathological Mineral Deposits at Various Sites, A. M. A. Arch. Path. **52**:458, 1951.

4. Gillman, J.; Gillman, T.; Mandelstam, J., and Gilbert, C.: Cytosiderosis and the Genesis of Arteriosclerosis and Other Fibrous-Tissue Reactions, Nature, London **159**:875, 1947.

5. Duff, G. L., and McMillan, G. C.: Pathology of Atherosclerosis, Am. J. Med. **11**:92, 1951.

6. Duff, G. L., in discussion on Paterson, J. C.: Reaction of the Arterial Wall to Intramural Haemorrhage, in Symposium on Atherosclerosis, Washington, D. C., National Academy of Sciences, National Research Council, Publication 338, 1954, p. 72.

Homeostasis of Connective Tissues

III. Magnesium-Sodium Equilibrium and Interactions with Strontium and Lead

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In previous publications * a basis has been laid for considering the ground substance of connective tissue as a heterogeneous system of colloid and water. At its simplest, the system would consist of a water-rich phase in equilibrium with a colloid-rich phase, both phases being in equilibrium with blood. In order to describe the latter equilibrium, it is necessary to know certain electrochemical properties of the colloidal aggregate as a whole and its interaction with the cations of blood. Among such intrinsic measurable properties of the ground substance are the density of the immobile colloidal (negative) charge and the free energies of formation of colloid-cation complexes. Nomograms showing the equilibrium of ground substance with blood sodium, calcium, and potassium have been derived from values for calcium-sodium and potassium-sodium exchange.† To complete the description of the equilibrium from the physiological standpoint, it is necessary to obtain data for the free energy of formation of the complex of ground substance with magnesium.

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* References 1 through 5.

† References 2 and 3.

The complete data may be used to formulate a description of the ground substance as an ion-exchange resin reacting with sodium, potassium, calcium, and magnesium. This information can be most conveniently expressed by a series of nomograms representing states in which any ion or pair of ions is displaced from the normal blood levels.

The same conditions that control the distribution of the physiological cations may be expected to apply to the reaction of ground substance with foreign metallic cations and with organic bases. The combination of a number of amines of biological importance with connective tissue colloid has been described,[4] and in the present communication data for lead and strontium binding are reported and are related to the biological effects of these ions.

METHODS

The method of liquid junction potential determination in connective tissues was used.[1] The experimental tissues were the skin and epiphysis of young adult rabbits. Two short 20-gauge needles were inserted, one subcutaneously into skin of the abdominal region as a reference junction, the other into the experimental tissue to be studied. Initially both needles were filled with approximately isotonic (0.15 M) NaCl (Solution I). These solutions were connected to indifferent saturated KCl calomel half-cells by means of saturated KCl-agar junctions and saturated KCl bridges. The potential difference was read, and the solution in the needle at the experimental junction was removed and renewed several times until successive readings agreed to within a millivolt. The isotonic saline at this junction was then replaced by Solution II, a one-tenth dilution (0.015 M NaCl), and the potential redetermined with successive changes of solution. Finally the initial base-line potential was restored by replacing Solution I in the needle. The displacement that occurs following application of 0.015 M NaCl has been termed the "first dilution potential," and it is denoted as E_A. Its magnitude depends on the base-binding capacity of the

TABLE 1.—*Dilution Potentials in Normal Rabbit Tissues*

Tissue	Ion	Molal Concentration	No. of Experiments	First Dilution Potential E_d, Mv.	Modified Dilution Potential E_d', Mv.
Skin	Mg	0.005	12	2.6	—2.4
	Sr	0.005	7	2.0	—2.2
	Pb	0.001	6	2.9	—1.1
Epiphysis	Mg	0.005	12	22.5	7.7
	Sr	0.005	7	19.7	4.9
	Pb	0.001	6	23.0	9.0

immobile connective tissue colloids; this quantity, expressed in equivalents per kilogram of tissue water, is denoted as x.

Solution III was an approximately isotonic saline solution containing a small quantity (0.005 M) of magnesium chloride (0.1425 M NaCl+0.005 M $MgCl_2$). After the restoration of the initial base-line potential with Solution I, the experimental needle was flushed and Solution I was replaced by Solution III. Reproducible readings were obtained with Solution III, differing only slightly from the initial potentials with Solution I. However, part of the colloid is bound by the magnesium of Solution III, so that application of Solution II now gives rise to a modified dilution potential, which is denoted E_d'. This is related to the modified density of colloidal charge, x', of the tissue following equilibration with the magnesium solution. The difference $(x—x')$ is taken as a measure of the bound magnesium complex. Strontium and lead binding were measured in essentially the same manner.

RESULTS

Dilution potentials on a series of 25 animals are presented (Table 1). These include values for E_d, the first dilution potential, and for E_d', the dilution potential modified by binding of the bivalent cation, magnesium, strontium, or lead.

DERIVATION OF EQUILIBRIUM CONSTANTS AND FREE ENERGIES OF EXCHANGE

It has been previously shown that the dilution potential is related to the density of immobile negative charges of the connective tissue, x, expressed as equivalents of base-binding capacity per kilogram of tissue water. When the tissue is equilibrated with a base such as calcium or magnesium, part of the immobile negative charge combines with the inorganic cations to form undissociated organic complexes. A part of the colloidal charge is thus neutralized, and the density of immobile negative charge is lowered to a new value, x'. The difference $(x—x')$ measures the equivalents of undissociated colloid-cation complex formed in the presence of the bivalent base. Following equilibration, we have seen that the modified dilution potential, E_d' becomes lower.

Two working equations are used, the derivation of which has been described.‡

The formula relating E_d and x is approximately linear:

$$E_d=-12.3+215x \qquad (1)$$

A similar relation holds between E_d' and x':

$$E_d'=-12.3+215x' \qquad (2)$$

The difference $(E_d—E_d')$ is therefore a measure of the bound cations following application of Solution III to the tissue. As previously explained,[1] the value —12.3 mv. is the limiting value of the dilution potential as x or x' approaches zero and is the approximate value of the liquid junction potential between Solutions I and II, or between Solutions III and II.

From the experimental determinations of E_d and E_d', the respective values of x, the initial, and x', the modified charge density, are computed from Equations (1) and (2) (Table 2). For each of the three ions, Mg, Pb, and Sr, and for the two tissues, skin and epiphysis, the ratio of x' to x is computed at the given ion concentration.

Estimation of the equilibrium constant for the formation of the ion-colloid complex

‡ References 1 and 5.

TABLE 2.—*Colloidal Charge Densities (Initial and Modified) and Equilibrium Constants for Rabbit Tissues*

Tissue	Ion	Initial Charge Density x (Eq. per Kg. H_2O)	Modified Colloid Charge Density x' (Eq. per Kg. H_2O)	Ratio x'/x	Equilibrium Constant, K
Skin	Mg	0.069	0.046	0.67	0.014
	Sr	0.067	0.044	0.64	0.011
	Pb	0.071	0.052	0.73	0.0042
Epiphysis	Mg	0.163	0.093	0.57	0.010
	Sr	0.149	0.080	0.54	0.004
	Pb	0.164	0.099	0.60	0.0026

TABLE 3.—*Comparison of Electrometric and Analytical Results in Cartilage*

	x (Eq. per Kg. H_2O)	r (Donnan Ratio)	$(Na^+)'$ mEq. per Kg. H_2O	(Mg^{++}) mEq. per Kg. H_2O	MgX, mEq. per Kg. H_2O	Total Mg, mEq. per Kg. H_2O	Equilibrium Constant, K
Epiphysis (electrometric)	0.163	1.68	252	2.8	22.8	25.6	0.010 *
Cartilage (direct analysis) †	.....	....	280	...	....	21.6	.....
Calculations from direct analysis	0.200	1.87	(280)	3.5	18.1	(21.6)	0.019

* Based on electrometric results for epiphyseal cartilage (Table 2).
† Results of Eichelberger and co-workers converted to milliequivalents per kilogram of H_2O.

assumes that reactions of the following type occur:

$$Me^{++} + Na_2X = MeX + 2Na^+$$

where Me^{++} denotes bivalent cation and X the colloid.

Then the equilibrium may be formulated as follows:

$$\frac{(Me^{++})^o r^2 x'}{x - x'} = K \qquad (3)$$

where $(Me^{++})^o$ is the molal concentration of the applied bivalent cation, r is the Donnan ratio, and x and x' the initial and modified colloidal charge densities. Appropriate substitutions in Equation (3) give values for the equilibrium constant K (Table 3).

The equilibrium constant is related to the standard free energy of the exchange reaction of Me^{++} with Na^+ by the formula

$$\triangle F^o = -RT \ln K$$

where R is the gas constant in calories per degree, T the absolute temperature and $\ln$ refers to logarithms to the natural base, e.

Standard free energies of formation of ion-colloid complexes for both physiological and nonphysiological ions appear in Table 4, which includes 10 ion pairs. Other combinations can be derived from these data.

TABLE 4.—*Free Energies of Formation of Ion-Colloid Complexes*

Ion Pair	Reaction	Standard Free Energy Change, $\triangle F^o$ Calories
Physiological ions		
Na^+-K^+	$2K^+ + Na_2X = K_2X + 2Na$	−2,500
Na^+-Ca^{++}	$Ca^{++} + Na_2X = CaX + 2Na^+$	−2,300
Na^+-Mg^{++}	$Mg^{++} + Na_2X = MgX + 2Na^+$	−2,700
K^+-Ca^{++}	$2K^+ + CaX = K_2X + Ca^{++}$	− 200
K^+-Mg^{++}	$Mg^{++} + K_2X = MgX + 2K^+$	− 200
Ca^{++}-Mg^{++}	$Mg^{++} + CaX = MgX + Ca^{++}$	− 400
Nonphysiological ions		
Pb^{++}-Na^+	$Pb^{++} + Na_2X = PbX + 2Na^+$	−3,500
Sr^{++}-Na^+	$Sr^{++} + Na_2X = SrX + 2Na^+$	−2,900
Pb^{++}-Ca^{++}	$Pb^{++} + CaX = PbX + Ca^{++}$	−1,200
Sr^{++}-Ca^{++}	$Sr^{++} + CaX = SrX + Ca^{++}$	− 600

COMMENT

(*A*) *Magnesium Equilibrium.*—The composition of the tissues in equilibrium with normal blood sodium and magnesium may be computed from the data. Normal blood sodium is taken as 150 mEq. per liter, and normal blood ionic magnesium as 1.0 mEq. per liter.[6] For epiphysis, the density of free colloidal charges, x, estimated from the first dilution potential, E_d, is 0.163 equivalents per kilogram of water. From this value, the Donnan ratio is estimated to be 1.68, which multiplied by normal blood sodium gives the value of tissue sodium as 252 mEq. per kilogram of water. Blood ionic magnesium multiplied by r^2 gives 2.8 mEq. as the value for tissue magnesium ions. From this value and that of the equilibrium constant, K, taken as 0.010, the quantity of bound magnesium is calculated to be 22.8 mEq. per kilogram of tissue water. Then the sum of ionic and bound magnesium is 25.6 mEq. per kilogram of water. These values may be compared with those obtained by direct analysis,[7] giving 280 mEq. for tissue sodium and 21.6 mEq. for total magnesium. Table 3 summarizes other comparisons of direct and indirect data. It is considered that the electrometric values are in satisfactory agreement with analytical results and that more exact agreement seems hardly to be expected because of the competition of other ions such as calcium and potassium. Results with about the same order of agreement were obtained for the calcium-sodium equilibrium.[2]

(*B*) *Equilibrium of Blood and Connective Tissue with Respect to Sodium, Potassium, Calcium, and Magnesium.*—From the present results and from previous studies on calcium-sodium and potassium-sodium equilibria, it has been concluded that the distribution of ions between blood and connective tissues follows the Gibbs-Donnan principle and that the chemical potentials of all the diffusible electrolytes are constant throughout the system. To represent these multiple and simultaneous changes of related variables, it is convenient to employ d'Ocagne nomograms analogous to those applied by Henderson in his studies of blood.[8] In a given nomogram, two independent variables are chosen. The physiochemical state is then described in terms of these two variables and any number of dependent variables which are known functions of the given two. As a first approximation, connective tissue may be treated as a system whose state is determined by five independent variables; these include the concentrations of the four blood cations, sodium, potassium, calcium, and magnesium. Given these four concentrations, the equilibrium is determined by x, the density of immobile colloidal negative charge expressed as equivalents per kilogram of tissue water. When these 5 independent variables are arranged in pairs, 10 combinations result with 2 of the variables in each combination taken as independent, the other 3 being held constant. The 10 combinations are listed in Table 5, which is an index of 10 nomograms describing the physiochemical system.

TABLE 5.—*Index of Nomograms (Figures 1 to 6)*

Figure No.	Independent Variables *	Constants *
1	x, Na	Ca, K, Mg
2	x, K	Na, Ca, Mg
3	x, Ca	Na, K, Mg
4	x, Mg	Na, Ca, K
5*A*	Na, K	x, Ca, Mg
5*B*	Ca, K	x, Na, Mg
5*C*	Na, Ca	x, K, Mg
6*A*	Na, Mg	x, K, Ca
6*B*	K, Mg	x, Na, Ca
6*C*	Ca, Mg	x, Na, K

* Ions refer to blood levels. The 10 nomograms include all pairs of the 5 variables.

The first 4 pairs of variables include x, the colloidal charge density, in addition to each of the 4 blood cations, so that 4 of the 10 nomograms include x as an independent variable (Figs. 1-4). These diagrams show that the distribution of electrolytes in the connective tissue is related to

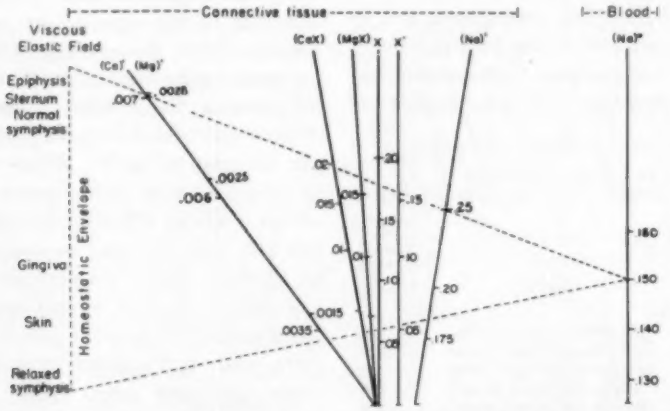

Fig. 1.—Nomogram of sodium equilibrium at varying colloidal states of aggregation. Variables are tissue cations, bound calcium and magnesium, blood sodium, and density of colloidal charge, x, in equivalents per kilogram of tissue water. Blood potassium, calcium, and magnesium are assumed constant at normal homeostatic levels. Varying states of colloidal aggregation are included, ranging from highly relaxed symphysis to epiphysis. Homeostatic envelope encloses all intermediate states in equilibrium with normal blood sodium, calcium, magnesium, and potassium.

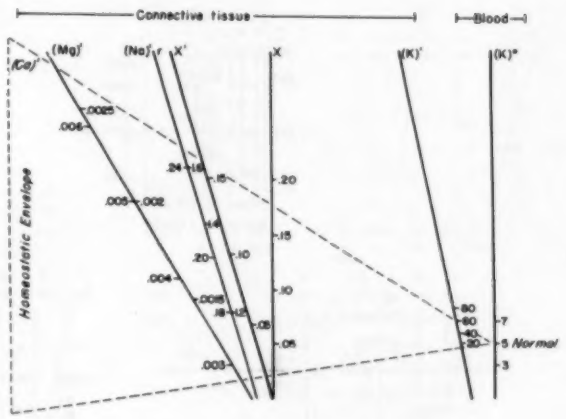

Fig. 2.—Nomogram of potassium equilibrium between blood and connective tissues. Independent variables: colloidal charge density, x, and blood potassium. Dependent variables: tissue potassium, sodium, calcium, and magnesium.

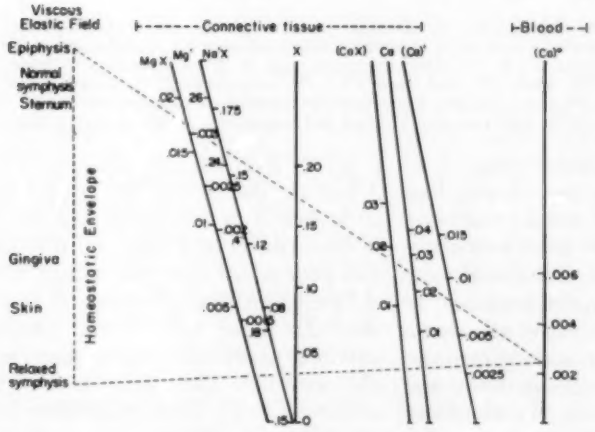

Fig. 3. — Nomogram of calcium equilibrium between blood and connective tissues. Independent variables: colloidal charge density, x, and blood ionic calcium. Dependent variables: tissue cations.

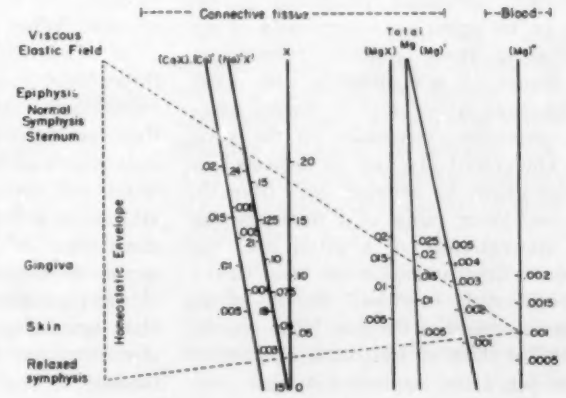

Fig. 4.—Nomogram of magnesium equilibrium between blood and connective tissues. Independent variables: colloidal charge density, x, and blood magnesium. Dependent variables: tissue cations.

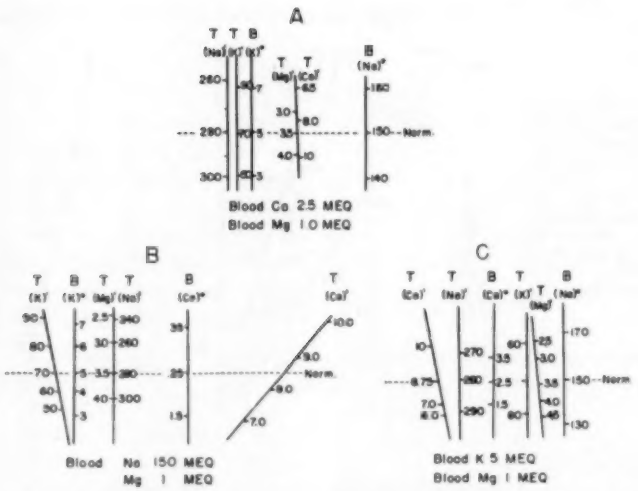

Fig. 5.—Ionic exchange nomograms for cartilage (normal monkey sternum). Blood magnesium constant. Horizontal dotted lines represent normal homeostatic distribution of ions between blood and cartilage. Eight variables are represented as follows: sodium, potassium, calcium, and magnesium in blood (B) and tissue (T). All concentrations expressed in milliequivalents per kilogram of water. In each nomogram the independent variables are two of the blood ions, the other two being held constant. Calcium and magnesium are the ionized fractions.

the colloidal charge density. Since x is directly proportional to the base-binding capacity of the tissue, it follows that as x increases in the connective tissue continuum the concentration of all the diffusible cations, both free and combined, also increases. It should be noted that high values of x occur in relatively dense tissues, such as cartilage and unrelaxed pubic symphysis, while low values of x are characteristic of such tissues as relaxed symphysis, skin, and the like. These states correspond further to stronger or weaker mechanical properties of the tissue, or to higher or lower levels of aggregation of tissue colloids, respectively. Body tissues, all in equilibrium with blood, have therefore wide limits of physical properties, depending essentially on the value of x. This variability may be indicated on the nomograms by drawing lines from the upper and lower values of x to the normal blood concentration of a given ion. The resultant triangular region has been termed the "homeostatic envelope," and it defines, for normal values of the four blood cations, the possible states of electrolyte equilibrium between blood and connective tissues.

In the six remaining nomograms (Figs. 5 and 6) the density of colloidal charge, x, is treated as a constant, and the result of displacement of a blood ion is examined. Calculations are made for a high value of x (sternal cartilage or epiphysis) at a point near the upper limit of the homeostatic envelope, where tissue cation concentrations also reach their upper limits. In three nomograms (Fig. 5) blood magnesium is held constant, and in three others (Fig. 6) it is variable. Where blood magnesium is held constant, one other blood cation is also held constant. When blood magnesium is treated as an independent variable, one of the other three blood cations is taken as the other independent variable. It is evident from these six nomograms that normal homeostatic distribution of the four cations between blood and connective tissue requires that all four be at the normal level. This normal distribution of ions in the system corresponds to any of the horizontal dotted lines of the six nomograms. In each nomogram eight quantities are related, the four blood concentrations and the four tissue concentrations. In each instance, two blood levels

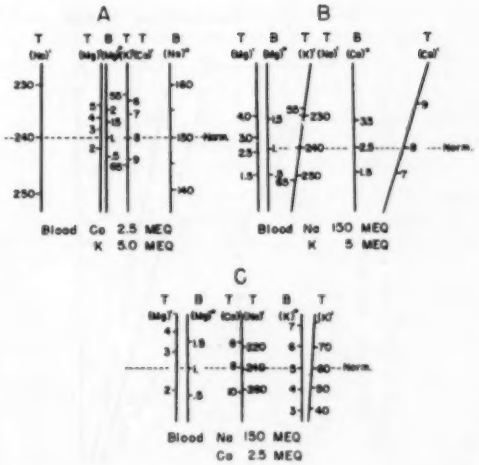

Fig. 6.—Ionic exchange nomograms for cartilage (normal rabbit epiphysis). Blood magnesium variable. Significance of variables same as in Figure 5.

are held constant, while the remaining six quantities are variable. If any two of these six quantities, in addition, are normal, then all eight concentrations will fall at the normal level. However, if one of the six variables is displaced from the normal there occurs a state in which four other variables are also displaced. Thus, in Figure 5*A*, the independent variables are the blood sodium and potassium. If blood potassium is elevated from normal when blood sodium, calcium, and magnesium are constant, it is found from the figure that tissue potassium is increased, whereas tissue sodium, calcium, and magnesium are decreased. The same result is found for any ion; elevation of the blood level of the given ion at constant blood levels of the other three results in an increase of the tissue level of the given ion and decreases in the tissue levels of the other three. This may be understood in terms of a generalized exchange reaction as formulated above (Table 4).

With magnesium, for example:

$$Mg^{++}+Na_2X=MgX+2Na^{+}$$
$$Mg^{++}+K_2X=MgX+2K^{+}$$
$$Mg^{++}+CaX=MgX+2Ca^{++}$$

Thus an increase of blood magnesium everywhere increases tissue magnesium and decreases tissue sodium, potassium, and calcium. Exchange reactions of the above type may be formulated for any pair of cations, either physiological or nonphysiological (e. g., strontium and lead).

The standard free energy change, ΔF°, for exchange reactions involving the physiological cations appears in Table 4. The data refer to six different ion pairs corresponding to the pairs of independent variables of six nomograms (Figs. 5 and 6; Table 5). The value of ΔF° for exchange of sodium and magnesium ions is based on a value of 0.012 for the equilibrium constant, K, which is a mean for several tissues. The calculations from which the nomograms are derived are based on the mean values for all tissues of the standard free energies of Table 4. Of the six values of ΔF°, three are independent; the other three, derived. The former are the free energies of formation of the complexes K_2X, CaX, and MgX from the sodium complex Na_2X. For potassium this value is —2500 cal.; for calcium it is —2300 cal. From these two values, it follows that the free energy of formation of K_2X from CaX is —200 cal. One may similarly obtain the free energies of formation of MgX from CaX or from K_2X.

From the construction of these nomograms, it is possible to infer a generalized form for any number of cations, either inorganic or organic,[4] in equilibrium between blood and connective tissues (Fig. 7).

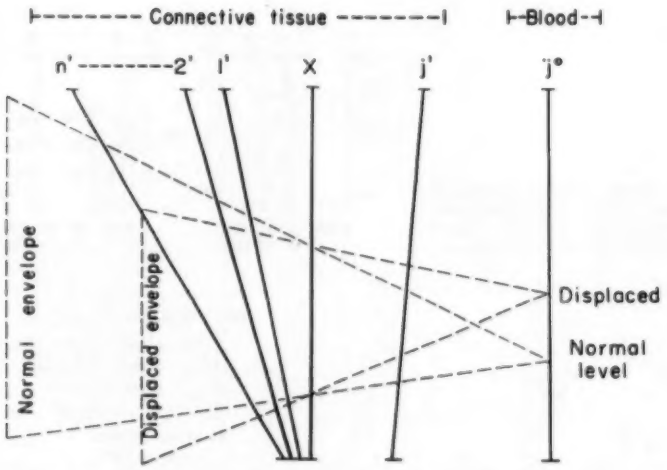

Fig. 7.—Generalized nomogram of equilibrium between blood and connective tissues. Independent variables: x, density of colloidal charge of connective tissue; $j°$, concentration of any blood cation. Dependent variables, tissue concentrations of cations, j', $1'$, $2'$ to n'.

(C) Binding of Lead and Strontium.—Because of their charged colloidal structure, connective tissues have the potentiality of concentrating metal cations. To this property is due the approximately 7-fold concentration of calcium, and 10-fold increase of magnesium found in cartilage, as compared with serum, as well as the accumulation of sodium and potassium.§ It is now shown that both lead and strontium have strong tendencies to displace calcium from connective tissue colloids. The free energy of exchange of lead with calcium is —1200 cal., and that of strontium with calcium is —600 cal. (Table 4). Accordingly, lead tends to displace strontium with a standard free energy change of —600 cal. The standard free energy changes involving other ion pairs may be readily derived from the data of Table 4. This strong affinity of lead for the ground substance would be expected to cause displacement of other metals and cations from their physiological associations and to produce a lead-colloid complex of such different properties as to disrupt the homeostatic state and to lead to new states of irritability and reactivity. Since magnesium also replaces calcium (ΔF——400 cal.), an excess of either magnesium or of strontium may produce comparable effects, including the rickets associated with these elements.

In the clinical management of lead poisoning, calcium salts are sometimes administered, and the effectiveness of this treatment may depend on ion-exchange reactions in which bound lead is replaced by calcium. From theoretical considerations, the limitations of this treatment may be understood, for infusion of calcium must lead to displacement of other cations as well, thus disturbing homeostasis, while releasing lead. The use of hormones, for example, parathyroid extract, affects the colloidal state of the ground substance, particularly of bone. Since the disaggregated matrix binds smaller quantities of cations, some lead may be released into the blood stream and excreted; yet the elevation in blood level may also result in an increase in total lead in other tissues. Evidence for these possibilities may be drawn from the clinical observations of Aub and his colleagues.[9]

In general, when fluids or electrolytes are introduced into the blood a stress is placed on homeostasis in the extracellular ground substance and there ensues a displacement, which is in the first case physicochemical,

§ References 2, 3, and 7.

and which can be described nomographically. Further equilibria at the physicochemical level would be expected to involve the intracellular colloids.

SUMMARY

The capacity of connective tissue to interact with the bivalent cations, magnesium, strontium, and lead, was studied by the method of liquid junction potentials. Tissues used were abdominal skin and epiphysis of the rabbit. Dilution potentials with one-tenth isotonic saline were measured before and after equilibration of the tissues with isotonic saline containing a low concentration of the bivalent cation, and the difference provided a measure of the amount of bivalent cation bound to immobile, negatively charged tissue colloid. Data for magnesium complete a description of the equilibrium of the physiological ions between blood and connective tissues. Reasonable agreement of electrometric values and those derived by direct analysis indicate that the Gibbs-Donnan theory may be used to describe this equilibrium. From the data, equilibrium constants and free energies of formation of metal-colloid complexes of the tissues were calculated. Values of the latter are as follows: for magnesium, —2700 cal.; for strontium, —2900 cal.; for lead, —3500 cal. Thus the foreign cations, and, in particular, lead, have a strong affinity for ground substance and would be expected to displace the physiological cations. The implications of this are discussed in relation to homeostasis and to treatment of lead poisoning.

REFERENCES

1. Joseph, N. R.; Engel, M. B., and Catchpole, H. R.: Interaction of Ions and Connective Tissue, Biochim. et biophys. acta **8**:575-587, 1952.

2. Engel, M. B.; Joseph, N. R., and Catchpole, H. R.: Homeostasis of Connective Tissues: I. Calcium-Sodium Equilibrium, A. M. A. Arch. Path. **58**:26-39, 1954.

3. Joseph, N. R.; Engel, M. B., and Catchpole, H. R.: Homeostasis of Connective Tissues: II. Potassium-Sodium Equilibrium, A. M. A. Arch. Path. **58**:40-58, 1954.

4. Joseph, N. R.; Engel, M. B., and Catchpole, H. R.: Combination of Biologic Amines with Connective Tissues, Am. J. Physiol. **179**:649, 1954.

5. Catchpole, H. R.; Joseph, N. R., and Engel, M. B.: Action of Relaxin on the Pubic Symphysis of the Guinea Pig, Studied Electrometrically, J. Endocrinol. **8**:377-385, 1952.

6. Standard Values in Blood, in Handbook of Biological Data, edited by E. C. Albritton, Washington, D. C., National Research Council, 1951, Vol. 1.

7. Eichelberger, L.; Brower, T. D., and Roma, M.: Histochemical Characterization of Inorganic Constituents, Connective Tissue and Chondroitin Sulfate of Extracellular and Intracellular Compartments of Hyaline Cartilages, Am. J. Physiol. **166**: 328-339, 1951.

8. Henderson, L. J.: Blood, New Haven, Conn., Yale University Press, 1928.

9. Aub, J. C.; Fairhall, L. T.; Minot, A. S., and Reznikoff, P.: Lead Poisoning, Medicine **4**:1-250, 1925.

Cardiac Cirrhosis

Report of a Case in a Six-Week-Old Infant with Congenital Malformation of Heart and Great Vessels

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Cardiac cirrhosis is a well-recognized pathologic entity.* Although the etiology of this disease has yet to be resolved in a satisfactory manner, the role of chronic passive hyperemia has been the subject of many papers. Clinically, both congestive heart failure and hepatic insufficiency need attention, but the first is usually treated and the second goes unrecognized in most instances. The purpose of this paper is to report the case of a 6-week-old infant dying with congestive heart failure, who was recently brought to autopsy at the Colorado General Hospital, since she was the youngest patient with the shortest duration of chronic passive hyperemia associated with cardiac cirrhosis ever observed personally or recorded in the American literature.

REPORT OF CASE

A 32-day-old white girl was admitted to the Colorado General Hospital because of congestive heart failure. The mother's pregnancy, labor, and delivery were uneventful. The birth weight was 9 lb. 1 oz. (4110 gm.). Physical examination after birth was thought unremarkable except for slight edema of the feet. The infant did well in the newborn nursery and was discharged home at 7 days of age. At 13 days of age the infant was brought to the referring physician's office because of "fever, cyanosis, and edema of the lower extremities" for 12 hours. Physical examination revealed an enlarged heart with a precordial systolic murmur not previously heard, an enlarged liver, and 4+ edema of the feet and legs. The tentative diagnoses were congenital heart disease—interatrial septal defect, congestive heart failure, and bronchopneumonia. Roentgenograms of the chest showed an enlargement of the heart, which appeared to be primarily right-sided. The patient improved on antibiotic therapy and digitalis and was referred to the Colorado General Hospital for a diagnostic work-up of the congenital heart disease. On admission the patient was a well-developed, well-nourished white baby girl, with respirations of 58 per minute. She showed severe dyspnea and generalized cyanosis when out of oxygen. The temperature was 37 C (98.6 F), pulse rate 110 per minute, and weight 3350 gm. A grade 3, harsh-blowing systolic murmur was heard along the left sternal border and over the precordial area. The heart was enlarged to both left and right by percussion. The liver was palpated 3 cm. below the costal margin in the right midclavicular line, and the spleen was palpated 2 cm. below the left costal margin in the midclavicular line. Pitting edema of the lower extremities was 3+. The femoral pulsations were good. An ECG showed right axis deviation. During her course in the hospital the patient became somewhat dusky, even though oxygen was administered continuously. Digitalis to the point of intoxication had no effect on the tachycardia. Additional roentgenograms of the chest were not revealing. The only blood chemistry values recorded were as follows: sodium, 146 mEq. per liter; potassium, 4.1 mEq. per liter; NPN, 30.5 mg. per 100 cc.; total protein, 4.9 gm. per 100 cc.; albumin, 3.5 gm. per 100 cc., and globulin, 1.4 gm. per 100 cc. The patient was apparently doing well when she was found dead in bed on the morning of the 15th day of hospitalization. Cardiac evaluation had not been completed at the time of death.

AUTOPSY FINDINGS

Gross Examination.—The autopsy was performed five and one-half hours after death. The body measured 60 cm. in length and weighed 3290 gm. External examination was normal except for enlargement of the liver to 5 cm. below the right costal margin, a 1+ pitting edema of the legs and feet, and looseness of the skin over the legs. Approximately 15 cc. of clear yellow fluid was present

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* References 1 through 10.

Fig. 1.—Heart. Note the hypertrophy of the ventricular muscle. The probe in the ventricles is placed through the interventricular defect. The upper probe lies in the coarcted segment of the aorta. × ⅓.

in each pleural cavity. The heart (Fig. 1) lay transversely in the thoracic cavity and measured 7 cm. in greatest width. The transverse diameter of the thoracic cage at this level measured 11.5 cm. The right atrium, right ventricle, and left ventricle were dilated and hypertrophied. The right ventricular musculature measured 5 mm. in greatest thickness; the left, 8 mm. The tricuspid valve was normal. The foramen ovale was patent to a probe inserted from the right atrium. The membranous portion of the interventricular septum consisted of a membrane 4 mm. in diameter containing a 1- to 2-mm. opening at its center. The interventricular septum was 6 mm. thick. The cusps of the aortic valve were thickened, and the aortic valve was relatively stenotic, measuring 19 mm. in circumference. The great vessels were abnormal. The pulmonary artery was dilated, measuring 27 mm. in circumference. The aorta measured only 18 mm. in circumference. The aorta was narrowed to 4 mm. in diameter from a point just distal to the common carotid artery to the origin of the left subclavian artery, whereas the major vessels from the arch of the aorta averaged 6 mm. in diameter. The descending thoracic aorta was 10 mm. in diameter distal to the narrowed segment. The ductus arteriosus was 4 mm. in length, and the internal diameter was 2 mm. The right lung weighed 60 gm., and the left 55 gm. (normal expected weights, 32 and 28 gm.). Surface and cut section were subcrepitant and mottled blue-red. The pulmonary veins were normal. The spleen weighed 21 gm. (normal expected weight, 13 gm.) and was firm and dark red. The liver weighed 205 gm. (twice the expected weight). The sharp anterior edge was 5 cm. below the costal margin in the right midclavicular line, and 7 cm. below the xiphoid process. The capsule of the liver was smooth and glistening and showed no gross nodularity. The surface was yellow-tan over the right lobe and yellow-tan with a slight green tinge over the left lobe. Cut sections revealed increased consistency of the entire liver, but more in the right lobe. The same coloration noted on the surface was seen on cut section. The portal vein,

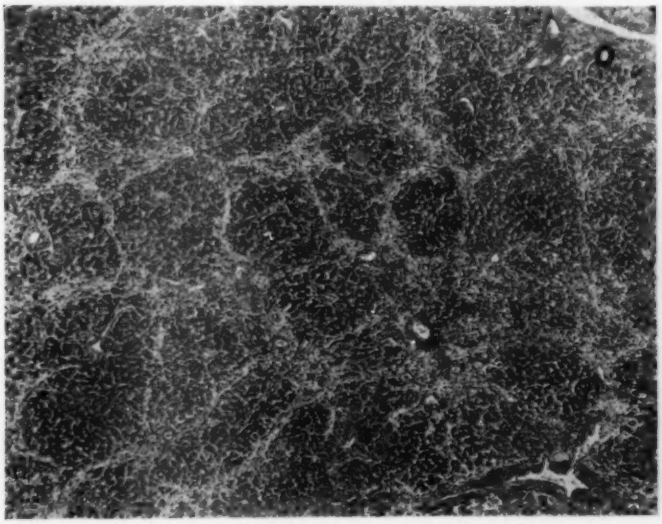


Fig. 2. — Liver. This photograph demonstrates the connection of central veins by connective tissue. Hematoxylin and eosin; reduced slightly from mag. × 30.

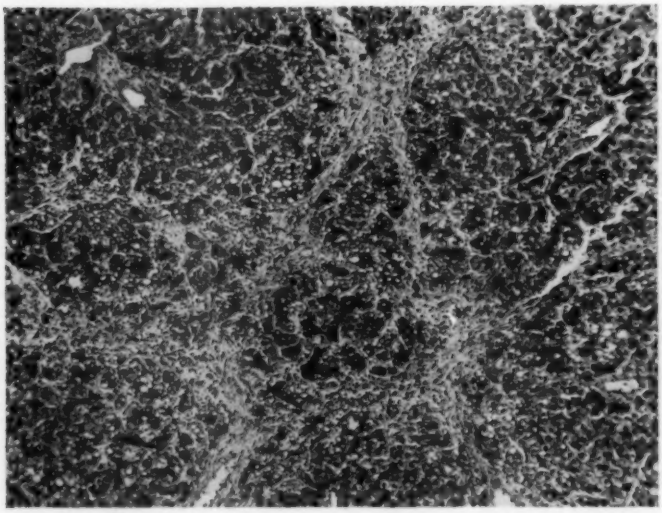


Fig. 3.—Liver. A higher magnification from an area in Figure 2. Gomori trichrome; reduced slightly from mag. × 90.

hepatic veins, and inferior vena cava showed no gross abnormalities. The gall bladder contained approximately 5 cc. of light green-brown, thin bile. Pressure on the gall bladder caused an immediate flow of bile from the ampulla of Vater. The remainder of the gross autopsy findings, including examination of the brain, was unremarkable.

Microscopic Examination.—Pertinent microscopic findings were limited to the liver (Figs. 2-5) and great vessels. The liver showed moderate infiltration of fat presenting as vacuoles in the cytoplasm of liver cord cells displacing the nuclei to the periphery. The distribution of these fat-laden cells was in no particular pattern. The central veins were engorged. Central zone liver cells were either fragmented or absent in many lobules, without inflammatory reaction. Connective tissue was prominently increased around cen-

Fig. 4.—Liver. This photomicrograph demonstrates the condensation of the reticulum fibers around central veins and connection of central veins. Reticulum stain; reduced about ¼ from mag. × 90.

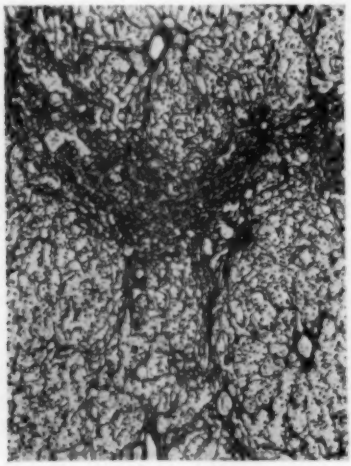
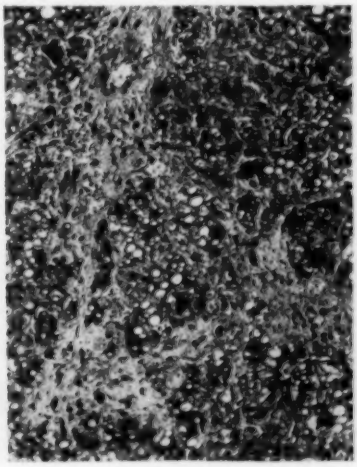


Fig. 5.—Liver. Note the connective tissue connecting adjacent central veins and the fatty infiltration of liver cells. Hematoxylin and eosin; reduced about ¼ from mag. × 180.



tral veins of lobules and around large hepatic veins. The connective tissue radiated from central veins, occasionally connecting with central veins of other lobules and rarely with the portal triads. In some areas proliferated bile ducts and blood-containing capillaries were prominent in the increased connective tissue, but inflammatory cells were inconspicuous. Bile casts were seen in bile canaliculi in all parts of lobules, but not in the large bile ducts in portal triads. The portal triads and blood vessels other than noted above were intact. No evidence of nodular regeneration of liver cells was seen. The central Kupffer cells and histiocytes in the new connective tissue contained brown pigment, all positive for iron with the Turnbull-blue method.

The wall of the coarcted segment of the aorta was thinned. The intima was irregularly slightly thickened by connective tissue. Elastic fibers were decreased in number and disorganized by abundant basophilic material between fibers. The intima of the ductus arteriosus was thickened by proliferated fibrocytes and showed loss of elastic fibers from the media. The cusps of the aortic valve were thickened by fibrous connective tissue focally loosened by basophilic mucoid material. The only other microscopic finding of note was chromatolysis of neurons of the dentate nucleus of the cerebellum. The final anatomic diagnoses were

1. Malformation, congenital, heart and great vessels
   (*a*) Interventricular septal defect
   (*b*) Hypertrophy and dilatation, heart
   (*c*) Patent ductus arteriosus
   (*d*) Coarctation of the aorta
   (*e*) Fibrosis, aortic valve
2. Fibrosis, liver (cardiac cirrhosis)
3. Infiltration, fat, liver
4. Hyperemia, acute and chronic, passive, lungs
5. Hydrothorax, bilateral
6. Edema, legs
7. Congestive heart failure—clinical diagnosis

COMMENT

In the following paragraphs the incidence, terminology, pathology, pathogenesis, relationship of duration of hyperemia and cardiac cirrhosis, clinical aspects and possible role of increased anoxia at high elevation are discussed.

Koletsky and Barnebee [1] stated that approximately 10% of patients with chronic congestive heart failure show cardiac cirrhosis at autopsy. Of these, 50% were associated with rheumatic heart disease. On the other hand, the incidence of cardiac cirrhosis in patients with rheumatic heart disease was 19%. Kotin and Hall [2] gave figures comparable to those of Koletsky and Barnebee. Garvin [3] set the incidence of cardiac cirrhosis at 4.4% (35 out of 790 cases of patients dying of cardiac disease). He further broke these figures down to indicate that 11.8% of the patients with rheumatic heart disease had cardiac cirrhosis, 5.3% of patients with hypertensive cardiovascular disease, and only 1.7% of patients with other types of heart disease. He also stated that in only 5 of the 35 cases was the antemortem diagnosis of cardiac cirrhosis suspected or made. Katzin and co-workers [4] selected 286 cases of chronic passive hyperemia of the liver from 2000 consecutive autopsies. A total of 95 cases, or 33%, had increased connective tissue in the liver. By eliminating 15 of these cases with biliary tract disease, the incidence of increased connective tissue was still 28%. Of the 286 cases, 4% had cirrhosis. Boland and Willius [5] selected 75 cases of chronic passive hyperemia of the liver in which either repeated bouts of congestive heart failure or a single prolonged episode of congestive heart failure occurred. Five of these had cirrhosis, an incidence of 7%. Castberg [6] selected 84 cases in which heart failure was present for one month to more than five years. The incidence of cirrhosis was 2.4%, although 58% of the cases showed some degree of increase in connective tissue in the liver. Thus the incidence of cardiac cirrhosis ranges from 2.4% to 10%, depending upon the manner in which the cases are selected.

The pathology of cardiac cirrhosis has become confusing because authors divide cases into various degrees of fibrosis of the liver with the severest recorded as cirrhosis.

The results may vary with individual interpretation. Many objections have been raised to the use of the term cirrhosis, but I believe that substitution of the term fibrosis is not the ultimate answer. Fibrosis has a very limited meaning. The term cirrhosis has been injudiciously used, but does, however, connote, if only by its common use through the years, more of the general processes of fibrosis, bile duct proliferation, infiltration of variable types and quantities of inflammatory cells, and distortion of lobular pattern as seen in the pathologic process under consideration. An interesting observation made by Katzin and co-workers [4] and confirmed by the cases of Castberg [6] is that fibrosis in chronic passive hyperemia is not always purely central. Katzin and co-workers stated that only 26 of 95 cases had pure central fibrosis. Castberg indicated that with severe fibrosis in chronic passive hyperemia, increase in connective tissue in the periportal spaces is frequent. As noted below, he has classified his cases into moderate fibrosis, severe fibrosis and cirrhosis, defining the last as fibrosis comprising over one-half the lobular area. This rather exacting criterion for the diagnosis of cardiac cirrhosis may well indicate why the incidence of cirrhosis in his series is so low.

It is generally accepted that the initial stage in cardiac cirrhosis is chronic passive hyperemia followed by necrosis of central lobular cells. The subsequent formation of connective tissue is not clearly understood. Himsworth [7] concluded from the work of Cameron and Oakley [8] that some substance which caused fibrosis to develop is liberated from degenerated liver cells. Moschcowitz,[9] on the other hand, believes that the genesis of this fibrotic process of the liver is the same as in any necrotic area of any organ, namely, the formation of granulation tissue. He described the sequence of events as follows: (1) absorption of degenerated cells; (2) infiltration of lymphocytes, plasma cells, and segmented neutrophiles; (3) transformation of lymphocytes into fibroblasts and then capillarization of the foci. This interpretation is difficult to accept in view of findings of the case reported. Sherlock [10] described all phases in the evolution of cardiac cirrhosis based on 41 liver biopsies from patients with cardiac failure. The most constant finding was central zone liver cell necrosis. In 15 of the biopsies, fatty change without constant pattern was present. After central zone necrosis, reticulum fibers became more closely approximated. This was followed by formation of new reticulum fibers and collagen. Sherlock did not describe proliferation of fibroblasts in this lesion. The more severe stages showed bands of connective tissue linking the central veins. In the long-standing cases, fibrosis, round-cell infiltration, bile duct proliferation, and nodules of regeneration of liver cells were observed. This latter picture could well be confused with portal cirrhosis. Sherlock also favored the interesting theory that collagen fiber formation occurs by precipitation in protein-rich exudate. This theory is not new. Eppinger and co-workers [11] referred to Rössle (1930), who perceived transformation of "exudate" of serous inflammation of the liver into connective tissue fibers. Baitsell,[12] in 1915, thought that connective tissue fibers could originate through transformation of fibrin. Currently several authors have reiterated some of these concepts in the genesis of the formation of collagen fibers. The reader is referred to the Josiah Macy, Jr. Foundation Conferences on Connective Tissue (1950-1955)[13] for more detailed information on collagen formation.

Most authors do agree that severity and quantity of increase in connective tissue in the liver is proportional to the duration of the congestive heart failure. In Koletsky and Barnebee's [1] cases, cardiac failure was present 1 to 14 years. Katzin and co-workers [4] stated that approximately 50% of the patients with chronic congestive heart failure for 9 months or longer will show morphologic increase in connective tissue in the liver. In Boland and Willius' [5] series, four to five months was the shortest duration that congestive heart failure was present. Castberg [6] selected cases in which chronic passive

hyperemia was present from one month to more than five years. He did not indicate whether any patients with the shorter periods of hyperemia showed the more extensive fibrosis. He did, however, state that the amount and severity of fibrosis increases with the duration of chronic passive hyperemia. Boland and Willius [5] made the interesting statement that "cases of congestive failure of short duration were excluded (from their series) as cardiac cirrhosis is not encountered in such instances." They based this opinion on a review of 500 unselected cases of chronic passive hyperemia of the liver. That the above quotation is most usually true is not questioned, but the case reported here indicates that it is by no means always true. Most authors also agree that cirrhosis is more likely to occur with repeated bouts of congestive heart failure than with single prolonged episodes of congestive heart failure. However, no quantitative comparison of the actual duration of the congestive heart failure, whether in intermittent episodes or progressive, has been made. The role of rheumatic heart disease has been emphasized, but it would appear that the increased incidence of cardiac cirrhosis associated with rheumatic heart disease is due to the prolonged cardiac failure that frequently occurs in this disease. Kotin and Hall [2] had 24 cases of congestive heart failure secondary to congenital heart disease, but only one of these patients showed cardiac cirrhosis, a woman, 56 years of age.

The clinical aspects of cardiac cirrhosis will not be discussed except to state that the problem of concomitant congestive failure usually far overshadows the cirrhosis, if present. It does seem possible, however, that the congestive heart failure can be overcome, but that the irreversible cirrhosis which has resulted will ultimately cause the demise of the patient. Johnson [14] enumerated several things that should make the clinician consider the diagnosis of cirrhosis in patients with congestive heart failure. These are as follows: (1) loss of appetite, loss of strength, loss of true body weight; (2) anemia and jaundice, both infrequent; (3) a small liver in the presence of congestive failure or enlarged liver after the failure has been relieved; (4) enlarged spleen; (5) unusually high venous pressure; (6) low plasma albumin, and (7) ascites greater than the peripheral edema, or persistence of ascites after peripheral edema has subsided, or ascites which does not respond to the usual therapeutic regimens.

Keller and Nute [15] reported a case (Case 5) of an infant who first had signs of congestive heart failure at 9 days of age. Hepatomegaly and splenomegaly were noted 17 days before death, which occurred at 4 weeks of age. Ascites and jaundice were noted seven days before death, with the jaundice most intense two days before death. There was no evidence of infection. The pathological findings were (1) saccular dilatation of a patent ductus arteriosus; (2) hypertrophy and dilatation of the heart; (3) thrombi in Thebesian veins and in both cardiac ventricles; (4) thrombi in abdominal aorta and several branches of the mesenteric artery; (5) chronic passive congestion of the liver with striking loss of hepatic parenchyma, fibrosis, and calcification. This case is too involved to be regarded simply as a case of cardiac cirrhosis. Calcification of the liver in cirrhosis is not described, to my knowledge. Although no photographs or detailed descriptions of this case are given by the authors, it is reasonable to suspect that the fibrosis and calcification in the liver may represent healing of infarcts, especially with the finding of thrombi in many arteries and in the heart.

The case herein reported is unusual, since it represents the earliest age and shortest duration of chronic passive hyperemia associated with cirrhosis known to me or found in the American literature. The degree of fibrosis which is assumed to have occurred after birth is striking. Chronic passive hyperemia of the liver appears to be the only obvious antecedent process. This case thus demonstrates that cardiac cirrhosis is not necessarily a disease which occurs only in prolonged single attacks of cardiac failure or

with repeated attacks of cardiac failure. This case points to another problem. In this era of extensive surgical treatment of patients with congenital heart disease, some of whom have had severe chronic passive hyperemia for long periods of time, the presence of cirrhosis and whether it be a factor in the eventual life expectancy may become an annoying clinical problem. However, a cursory review of the patients who died of congenital heart disease at the Children's Hospital of Pittsburgh † revealed no similar cases.

An incidental thought in the case reported has reference to the locale in which the infant was born. Perhaps anoxia occurring with chronic passive hyperemia is greater in Pueblo, Colo., elevation 4668 feet above sea level, than in areas of lower elevation. The mean peripheral arterial oxygen tension in a study of normal adults [16] acclimatized at an elevation of one mile is 74.8 mm. Hg. The mean peripheral arterial carbon dioxide tension is 33 mm. Hg. The corresponding figures [17] at sea level are peripheral arterial oxygen tension 100 mm. Hg; peripheral arterial carbon dioxide tension 47 mm. Hg. Studies of effect of altitude and, hence, changes of arterial oxygen tension with regard to the incidence of cardiac cirrhosis and comparable disease have not been carried out, to my knowledge.

SUMMARY

An unusual case of cardiac cirrhosis occurring in a 6-week-old infant with congenital malformation of the heart and great vessels is reported in detail, because this case represents the youngest person and the shortest duration of chronic passive hyperemia of the liver associated with cardiac cirrhosis seen personally or recorded in the American literature.

The incidence of cardiac cirrhosis varies from 2.4% to 10%, depending on how the cases are selected. Rheumatic heart disease is the commonest cause of cardiac failure in cases of cardiac cirrhosis.

The pathogenesis of cardiac cirrhosis remains controversial.

The pathologic picture in cardiac cirrhosis is one in which an apparent or real increase in fibrous connective tissue around central veins often connects central veins of adjacent lobules and occasionally portal triads. This connective tissue may contain proliferated bile ducts, some lymphocytes, and plasma cells.

Clinically the problem of cardiac failure overshadows the cirrhosis, if present. It seems quite possible that cardiac failure can be successfully treated, only to have the patient die of hepatic insufficiency, usually unrecognized and untreated.

The possibility of occurrence of cardiac cirrhosis with short periods of chronic passive hyperemia may well create a problem in patients with congenital heart disease who undergo surgery. These patients often have long-standing chronic passive hyperemia.

The peripheral arterial oxygen saturation at an elevation of approximately one mile is lower, and the peripheral arterial carbon dioxide tension is much higher, than at sea level. It is possible that at high elevation this greater anoxia may play an important role in development of cardiac cirrhosis.

† Fetterman, G. H.: Personal communication to the author, 1956.

REFERENCES

1. Koletsky, S., and Barnebee, J. H.: "Cardiac" or Congestive Cirrhosis, Am. J. M. Sc. **207**:421-430, 1944.

2. Kotin, P., and Hall, E. M.: "Cardiac" or Congestive Cirrhosis of Liver, Am. J. Path. **27**: 561-571, 1951.

3. Garvin, C. F.: Cardiac Cirrhosis, Am. J. M. Sc. **205**:515-518, 1943.

4. Katzin, H. M.; Waller, J. V., and Blumgart, H. L.: "Cardiac Cirrhosis" of the Liver: A Clinical and Pathologic Study, Arch. Int. Med. **64**: 457-470, 1939.

5. Boland, E. W., and Willius, F. A.: Changes in the Liver Produced by Chronic Passive Congestion, Arch. Int. Med. **62**:723-739, 1938.

6. Castberg, T.: Changes in the Liver Due to Chronic Passive Congestion, Acta path. et microbiol. scandinav. **30**:343-357, 1951.

7. Himsworth, H. P.: Lectures on the Liver and Its Diseases, Cambridge, Mass., Harvard University Press, 1950.

8. Cameron, G. R., and Oakley, C. L.: Transplantation of Liver, J. Path. & Bact. **38**:17, 1934.

9. Moschcowitz, E.: Morphology and Pathogenesis of Cardiac Fibrosis of the Liver, Ann. Int. Med. **36**:933-955, 1952.

10. Sherlock, S.: Liver in Heart Failure: Relation of Anatomical, Functional, and Circulatory Changes, Brit. Heart J. **13**:273-293, 1951.

11. Eppinger, H.; Kaunitz, H., and Popper, H.: Die seröse Entzündung, Wien, Springer-Verlag, 1935, pp. 72-75.

12. Baitsell, G. A.: Origin and Structure of a Fibrous Tissue Which Appears in Living Cultures of Adult Frog Tissues, J. Exper. Med. **21**:455-479, 1915.

13. Transactions of the Josiah Macy, Jr. Foundation Conferences on Connective Tissue, 1950-1955, New York, Josiah Macy, Jr. Foundation Publications, 1950-1955.

14. Johnson, C. S.: Cardiac Cirrhosis, New York J. Med. **53**:2811-2814, 1953.

15. Keller, P. D., and Nute, W. L., Jr.: Cirrhosis of the Liver in Children, J. Pediat. **34**:588-615, 1949.

16. Anderson, L. L.; Willcox, M. L.; Silliman, J., and Blount, S. G., Jr.: Pulmonary Physiology of Normal Individuals Living at an Altitude of One Mile, J. Clin. Invest. **32**:490-496, 1953.

17. Best, C. H., and Taylor, N. B.: Physiological Basis of Medical Practice, Ed. 3, Baltimore, Williams & Wilkins Company, 1943, pp. 530-535.

Production of Aortic Rupture in Rats Fed Purified Diets and beta-Aminopropionitrile

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Geiger and associates were the first to produce skeletal deformities by feeding sweet pea meal (Lathyrus odoratus) and casein to growing rats.[1] This observation has been confirmed by other workers.* Spontaneous aortic rupture was not described until the report of Ponseti and Baird.[4] To produce aortic rupture as well as the bony malformations, these authors fed sweet pea meal alone or supplemented with minimal concentrations of casein. We were able to produce aortic rupture in 50% of the rats only when the casein level was reduced to 10%.[5] Dasler was able to decrease the severity of bony deformity by increasing the protein concentration fed with the sweet peas.[6] Walker and Wirtshafer have observed more extensive arterial injury than other workers after feeding crude sweet pea meal.[7] When crude diets † were used, one could not resolve which dietary ingredients in casein modified the action of the toxic factor. The toxic factor in sweet peas has been identified as β-(N-γ-L-glutamyl)-aminopropionitril.[8] It has been demonstrated that feeding β-aminopropionitrile (BAPN) at levels of 0.15%-0.4% in the diet will produce skeletal deformities as readily as the incorporation of 50% crude sweet pea meal. Aortic rupture, however, has occurred only rarely following the feeding of BAPN.‡ In the present study, mixtures of dietary ingredients were fed in excess together with BAPN to determine whether they would enhance or suppress the development of lathyrism.

METHOD

Sprague-Dawley rats of either sex, weighing 39-55 gm., were used in these assays. Approximately similar diets were fed to which either fluid BAPN § (Experiment A) or the hydrochloride salt of BAPN (Experiment B) was added with amino acids alone or combined with an excess of certain B vitamins.

	Diet A	Diet B ‖
Casein (Borden's).....	12% Crude	12% "Vitamin-free"
Cerelose (dextrose)....	78%	78%
Salt (Wesson).....	4%	4%
Corn oil.....	6%	6%

In the control and test diets the water-soluble vitamins were added to and mixed with the casein-Cerelose-salt mixture (mg/kg.): thiamine HCl, 2.5; riboflavin, 3; pyridoxine HCl, 2.5; calcium pantothenate, 20; nicotinic acid, 10; i-inositol, 10; biotin, 0.5; folic acid, 2, and menadione, 10. The following were also added to the dry mixture (mg/kg.): choline chloride, 1000; DL-methionine, 1000, and L-cystine, 1000. The fat-soluble vitamins were diluted in 60 gm. of corn oil (per kilogram of diet): vitamin A acetate, 6000 I. U.; vitamin D, 60 I. U.; α-tocopherol, 100 mg. In test diets used in Experiment A, 2 ml/kg. of BAPN was suspended by agitation in the corn oil and fat-soluble vitamins before mixing with the dry ingredients. When supplements of amino acids were included in the diets, they were added at the expense of Cerelose. "Vitamin-free" casein was substituted for crude Borden's casein in Experiment B. In test

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* References 2-4.

† References 4-7.

‡ References 9-10.

§ Synthetized by E. D. Schilling with F. M. Strong, Department of Agricultural Biochemistry, University of Wisconsin.

‖ Vitamin-free casein, Wesson salt, amino acids, and vitamins were purchased from Nutritional Biochemical Corporation, Cleveland.

diets used in Experiment B the more stable hydrochloride of BAPN, 2.5 gm/kg., was mixed with the dry ingredients before the addition of oil and fat-soluble vitamins. The control and test diets were also supplemented with an excess of vitamins, DL-methionine, or glycine, as shown in Table 2. The diets were prepared every other week and kept at 0 to 5 C. Six to seven rats were housed in one mesh-bottom cage and fed ad libitum for seven weeks. When the rats died, or after the seventh week, autopsies were performed, and the following were recorded: weight gain; presence of sternal, vertebral column, or femoral deformities; aneurysm, hemorrhages, or rupture of the aorta; herniation or other obvious alterations. In this study a correlation between the gross abnormalities and their incidence in the different assays seemed more informative than a microscopic analysis of tissues in the evaluation of results.

EXPERIMENT A.—DL-tryptophan, L-arginine, glycine, DL-alanine, and L-glutamic acid were added alone or mixed with an excess of some B vitamins and incorporated with 2 ml/kg. of BAPN in the test diets. Observations made in two control and eight test assays are tabulated in Table 1.

In the two dietary control groups without BAPN (Assays 1 and 5), there was no evidence of skeletal deformity, herniation, or alteration in the aortic arch. The weight gain was the greatest in the two control assays. When 2 ml/kg. of BAPN was incorporated in the diet with an amino acid (Assays 6, 7, and 9) or an amino acid combined with an excess of certain B vitamins (Assays 2, 3, 4, 8, and 10), skeletal malformations developed. The deformities observed in the various assays were of equivalent severity. Herniation into the femoral canal or scrotum was relatively common in Assays 6 to 10. A fivefold excess (12.5 mg/kg.) of thiamine HCl and pyridoxine HCl when combined with glutamic acid improved the weight gain without influencing the development of hernia (Assay 10). In several instances the intestine became incarcerated in the hernia and the animals died of intestinal obstruction. On the other hand, hernias were not observed when an excess of certain vitamins and DL-tryptophan or L-arginine were fed with BAPN (Assays 2, 3, and 4). Rats fed an excess of alanine or glutamic acid (alone) gained poorly. Addition of thiamine HCl to alanine failed to improve the weight gain (Assay 8). The incidence of aortic rupture was relatively infrequent, because only 4 of 51 rats died of hemothorax and aneurysms of the aortic arch were observed only in 2 of the 47 survivors.

TABLE 1 (Experiment A).—*Incidence of Hernia and Aortic Rupture in Rats Fed BAPN and Certain Amino Acids for Forty-Nine Days*

Assay	Supplement Added to Diet A: Vitamin Excess, Mg./Kg.		Supplement Added to Diet A: Amino Acid, Gm./Kg.		BAPN, Ml./Kg.	No. of Rats	Weight Gain and Incidence of Changes: Gm./Day	Hernia	Aortic Rupture
1	.....	0.0	.....	0.0	0	6 (0)*	2.29	0	0
2	B mixture †	...	DL-tryptophan	10.0	2‡	7 (2)	1.58	0	1
3	B1 B6	5.0 5.0	DL-tryptophan	10.0	2	7 (1)	1.70	0	1
4	B6	5.0	L-arginine	10.0	2	7 (3)	1.55	0	1
5	.....	0.0	.....	0.0	0	6 (1)	2.21	0	0
6	.....	0.0	Glycine	13.0	2	6 (1)	1.56	2	0
7	.....	0.0	DL-alanine	17.0	2	6 (1)	1.27	2	1
8	B1	12.5	DL-alanine	17.8	2	6 (1)	1.02	3	0
9	.....	0.0	L-glutamic	29.4	2	6 (0)	1.26	2	0
10	B1 B6	12.5 12.5	L-glutamic	29.4	2	6 (2)	1.74	3	0

* Number of rats that died during the experiment is shown in parentheses.

† Vitamin B mixture (mg/kg.) = folic acid, 4; biotin, 1; riboflavin, 6; calcium pantothenate, 40; nicotinic acid, 20, and i-inositol, 20.

‡ Two milliliters of BAPN is equivalent to 1.9 gm. of BAPN.

EXPERIMENT B.—"Vitamin-free" casein was substituted for crude casein, and the more stable hydrochloride salt of BAPN was given. In Assays 1-8, DL-methionine supplement was increased to 0.8%, and the vitamin mixtures were varied. In Assays 9 and 10, the rats were fed 0.8% glycine with an excess of B vitamins. Observations on four control and six test groups performed on three separate occasions are shown in Table 2.

Rats on control diets gained more weight than those fed BAPN-HCl (Assays 1, 5, 7, and 9). BAPN-HCl is toxic at this concentration, because the average weight gain was always less in the test animals. Test rats supplemented with DL-methionine alone (Assay 2) gained less than those receiving B vitamins and methionine (Assays 3, 6, and 8). Herniation was observed in 2 of 48 test rats. Aortic rupture occurred in 19 of 36 rats fed DL-methionine alone or combined with one or more B vitamins (Assays 2, 3, 4, 6, and 8). The rats frequently first developed dyspnea and then died after four or more weeks. Aortic aneurysm or hemorrhage was observed in 8 of 17 surviving rats which were fed DL-methionine. Some alteration was observed in the aortic arch in 27 of 36 rats in the methionine test group. It is of interest that those rats which gained the most weight without severe vertebral column deformities and hindlimb weakness appeared most prone to aortic rupture (Assay 6). Rats receiving 0.8% glycine and an added maintenance level of B vitamins in excess with BAPN-HCl (Assay 10) were less active and gained less weight than rats in Assay 8. In Assay 10 one rat died of aortic rupture, one of hernia, and a third following hindlimb paralysis. Gross arterial hemorrhage or aneurysm was observed in four of nine surviving rats in Assay 10. Fecal impaction was also observed in three rats in this group.

TABLE 2 (Experiment B).—*Incidence of Aortic Rupture and Hernia in Rats Fed BAPN-HCl with* DL-*Methionine or Glycine for Forty-Nine Days*

	Supplement Added to Diet B						Weight Gain and Incidence of Changes		
Assay	Vitamin Excess, Mg./Kg.		Amino Acid, Gm./Kg.		BAPN HCl, Gm./Kg.*	No. of Rats	Gm./Day	Hernia	Aortic Rupture
1	.....	0.0	DL-methionine	8.0	0.0	6 (0)†	2.21	0	0
2	.....	0.0	DL-methionine	8.0	2.5	6 (2)	1.51	1	2
3	B mixture ‡	...	DL-methionine	8.0	2.5	6 (4)	1.66	0	4
4	B_{12}	12.5	DL-methionine	8.0	2.5	6 (3)	1.72	0	3
5	B mixture ‡	...	DL-methionine	8.0	0.0	6 (0)	3.01	0	0
6	B mixture ‡	...	DL-methionine	8.0	2.5	6 (5)	1.91	0	5
7	B mixture §	...	DL-methionine	8.0	0.0	6 (0)	2.28	0	0
8	B mixture §	...	DL-methionine	8.0	2.5	12 (6)	1.65	0	5
9	B mixture §	...	Glycine	8.0	0.0	6 (0)	2.54	0	0
10	B mixture §	...	Glycine	8.0	2.5	12 (3)	1.54	1	1

* Two and one-half grams of BAPN-HCl is equal to 1.7 gm. of BAPN.

† Number of rats that died during the experiment is shown in parentheses.

‡ Vitamin B mixture (mg/kg.) = folic acid, 10; biotin, 2.5; thiamine HCl, 12.5; riboflavin, 15; nicotinic acid, 50; calcium pantothenate, 100, and i-inositol, 50.

§ Vitamin B mixture (mg/kg.) = folic acid, 2; biotin, 0.5; thiamine HCl, 2.5; riboflavin, 3; pyridoxine HCl, 2.5; calcium pantothenate, 20; nicotinic acid, 10; i-inositol, 10.

COMMENT

In the present study 12% casein with a known concentration of BAPN was fed so that the addition of different amino acids could be evaluated for their influence on toxicity. It has not been possible to prevent malformation of bone in rats fed BAPN by the addition either of some amino acids or of B vitamins in these experiments. Peculiarly, however, associated changes, such as weight gain, herniation, or aortic rupture, have been modified by the supplements which are fed with BAPN. Weight gains were greatest in those test rats which received excess vitamins with one of the following: DL-tryptophan, L-glutamic acid, or DL-methionine. Herniation was more frequent in test rats fed glycine, DL-alanine, or L-glutamic without added vitamins. One might well anticipate that there would be some relationship between the development of herniation and aortic rupture. This, however, was not the case in the test assays. An excess of one or more B vitamins was fed with BAPN and amino acids in some of the assays. It was not possible to counteract BAPN toxicity

significantly by the addition of an excess of B vitamins. There is a suggestion, however, that added supplements of mixtures of B vitamins exert some influence on weight gain, and possibly hernia formation.

In a former study, when 1.9 gm/kg. of BAPN was added to the diet without supplements of amino acids or B vitamins, aortic rupture occurred in 2 of 20 rats.[10] In Experiment A of this study the incidence of rupture was approximately similar, since 4 of 51 rats died of hemothorax. In five test assays in Experiment B, when an excess of DL-methionine was fed with BAPN-HCl, the incidence of aortic rupture was greater, being 19 of 36. The microscopic alterations which developed in the aorta of rats fed BAPN are similar to the changes previously reported following the feeding of crude sweet pea meal.¶ Only one rat developed visible hemorrhage in the lower thoracic aorta, and no gross hemorrhages were observed in the abdominal aorta. The more widespread arterial injury observed by Walker and Wirtshafer,[7] which is in contrast to our findings, raises the question whether the crude diet fed or the Long-Evans strain of rat is responsible for the variations. These dietary studies offer no suggestion as to the mechanism by which BAPN exerts its influence on developing mesenchymal tissue. The assays indicate that BAPN is toxic, since the test rats always gained less weight than the control animals. Some of the manifestations of BAPN toxicity, such as weight gain, hindlimb muscle weakness, and aortic rupture, can be modified by an excess of B vitamins and some of the amino acids.

BAPN belongs to a family of chemicals many of which are known to be toxic.# In studies on nitrile toxicity, deformities in the skeleton had not been recorded until after the discovery of BAPN. The lethal dose and the response of the animal to a nitrile are extremely variable, depending upon the chemical structure.[12] Since the discovery of BAPN, only aminoacetonitrile and bis-β-(cyanoethyl)-amine have been added to the list of nitriles which are capable of producing skeletal malformation.* Hindlimb paralysis has been frequently observed in rats fed sweet pea meal. The paralysis has been attributed to compression of the cord secondary to deformities in the vertebral column.[3] This process undoubtedly contributes to the cause of paralysis in some rats. The observation of hindlimb spasticity or weakness in rats before vertebral column deformities develop suggests that the effect of BAPN on nerve tissue deserves further investigation.

¶ References 5 and 7.

References 11-13.

SUMMARY

A known concentration of BAPN was fed with different amino acids and vitamins in 14 test assays to determine whether they would influence or affect in any manner the activity of the toxic factor. Feeding BAPN in diets containing 12% casein with adequate salts, vitamins, fats, and carbohydrates to growing rats will produce aortic rupture, in addition to the other abnormalities which have been observed in lathyrism. Aortic rupture occurred in 5 of 63 test rats in nine assays when BAPN was fed with one of the following: DL-tryptophan, L-arginine, glycine, DL-alanine, and L-glutamic acid. In five assays when 8 gm/kg. of DL-methionine alone or mixed with an excess of certain B vitamins and BAPN was fed, aortic rupture occurred in 19 of 36 rats. None of the amino acid and vitamin mixtures fed with BAPN prevented the development of skeletal deformities. The incidence of aortic rupture, however, was modified by the amino acid which is fed with BAPN. DL-methionine predisposed to aortic rupture more than any other amino acid tested when fed with BAPN to young rats receiving 12% casein in the diet.

REFERENCES

1. Geiger, B. J.; Steenbock, H., and Parsons, H. T.: Lathyrism in the Rat, J. Nutrition **6**:427, 1933.

2. Robinson, J. J., and Bast, T H: Bone Changes Due to Lathyrism in Rats, Anat. Rec. **59**:283, 1934.

3. Lewis, H. B.; Fajans, R. S.; Esterer, M. B.; Shen, C. W., and Oliphant, M.: Lathyrism in the Rat, J. Nutrition **36**:537, 1948.

* References 9-10.

4. Ponseti, I. V., and Baird, W. A.: Scoliosis and Dissecting Aneurysm of the Aorta in Rats Fed with Lathyrus Odoratus Seeds, Am. J. Path. **28**:1059, 1952.

5. Bachhuber, T. E., and Lalich, J. J.: Effect of Sweet Pea Meal on the Rat Aorta, A. M. A. Arch. Path. **59**:247, 1955.

6. Dasler, W.: Partial Protection Against Odoratism (Sweet Pea Lathyrism) by Diets High in Gelatin and Casein, Proc. Exper. Biol. & Med. **85**:485, 1954.

7. Walker, D. G., and Wirtshafer, Z. T.: Histopathogenesis of Aortic Aneurysms in the Lathyrus Fed Rat, A. M. A. Arch. Path. **61**:125, 1956.

8. Schilling, E. D., and Strong, F. M.: Isolation Structure and Synthesis of a Lathyrus Factor from L. Odoratus, J. Am. Chem. Soc. **76**:2848, 1954.

9. Wawzonek, S.; Ponseti, I. V.; Shepard, R. S., and Wiedenmann, L. G.: Epiphyseal Plate Lesions, Degenerative Arthritis, and Dissecting Aneurysm of Rat Aorta Produced by Aminonitriles, Science **121**:63, 1955.

10. Bachhuber, T. E.; Lalich, J. J.; Angevine, D. M.; Schilling, E. D., and Strong, F. M.: Lathyrus Factor Activity of Beta-Aminopropionitrile and Related Compounds, Proc. Soc. Exper. Biol. & Med. **89**:294, 1955.

11. Rentz, E.: Zur Entgiftung der Nitrile, Arch. internat. pharmacodyn. **36**:455, 1929-1930.

12. Treon, J. F.; Dutra, F. R., and Cappel, J.: Toxicity of Di-(Acetyl cyanide), Arch. Indust. Hyg. **4**:573, 1951.

13. Bray, H. G.; Hybs, Z., and Thorpe, W. V.: Metabolism of Derivatives of Toluene, Biochem. J. **48**:192, 1951.

News and Comment

PERSONAL

Kovalenko Medal Awarded Dr. Peyton F. Rous.—At the annual meeting of the National Academy of Sciences held in Washington on April 23, 1956, Dr. Peyton F. Rous was awarded the Kovalenko Medal in recognition of his outstanding contributions to the medical sciences.

Dr. Frank B. Johnson Elected to Council of Histochemical Society.—Dr. Frank B. Johnson, Chief of the Histochemistry and Cytophysics Branch of the Armed Forces Institute of Pathology, was elected a member of the council of the Histochemical Society at its recent meeting in Atlantic City.

Appointments.—Dr. Howard T. Karsner has been elected Chairman of the Advisory Medical Board of the Leonard Wood Memorial for the Eradication of Leprosy.

Capt. William M. Silliphant, Director, Armed Forces Institute of Pathology, has been appointed to the Board of Editors of the American Journal of Clinical Pathology.

ANNOUNCEMENTS

Conference on Cancer.—The American Cancer Society and the National Cancer Institute of the Public Health Service will jointly sponsor the third National Cancer Conference in Detroit, June 4-6. More than a thousand research scientists and clinicians, including many from foreign countries, are expected to attend. The opening session, in the Sheraton-Cadillac Hotel, will feature addresses by Dr. John R. Heller, director, National Cancer Institute, Bethesda, Md., and Dr. Charles S. Cameron, scientific director, American Cancer Society, New York City. Morning and afternoon sessions will begin with a general session, at which a subject of broad interest will be presented by an outstanding speaker. The general sessions will then break into various symposiums to discuss cancer of various body sites. A host committee representing medical and health groups of the Detroit area, headed by Dr. Harry M. Nelson, Detroit, of the Southeastern Michigan Division, American Cancer Society, will arrange a public cancer forum for Tuesday evening. The last National Cancer Conference was held in Cincinnati in 1952.

Carl V. Weller Lectureship.—The Michigan Pathological Society has announced the establishment of the Carl V. Weller Lectureship in recognition of the many contributions made by Dr. Weller in the fields of pathology and clinical pathology. The first lecturer will be Dr. Howard T. Karsner, medical advisor to the Navy, Washington, D. C., and will be presented in Ann Arbor, Mich., on Dec. 8, 1956.

Public Health Service Research Grants.—The Public Health Service has announced a new procedure to expedite the processing of research grant applications for those requests which do not exceed $2000 plus indirect costs and which do not ask support for more than one year. Such applications will be accepted and processed on receipt and are not therefore subject to the usual deadlines for submission prior to review.

Council recommendations can be expected on these applications within one to four months from the time of submission. These procedures do not apply for requests for supplements to existing grants.

Address all applications as well as requests for forms or additional information to the Division of Research Grants, National Institutes of Health, Bethesda 14, Md.

Books

Embryologie: Ein Lehrbuch auf allgemein biologischer Grundlage. By Prof. Dr. Dietrich Starck, Direktor des Dr. Senckenbergischen Anatomischen Inst. der Univ. Frankfurt/Main, Germany. Price, $18.55. Pp. 688, with 522 illustrations. Georg Thieme Verlag, Herdweg 63, Stuttgart, Germany, 1955.

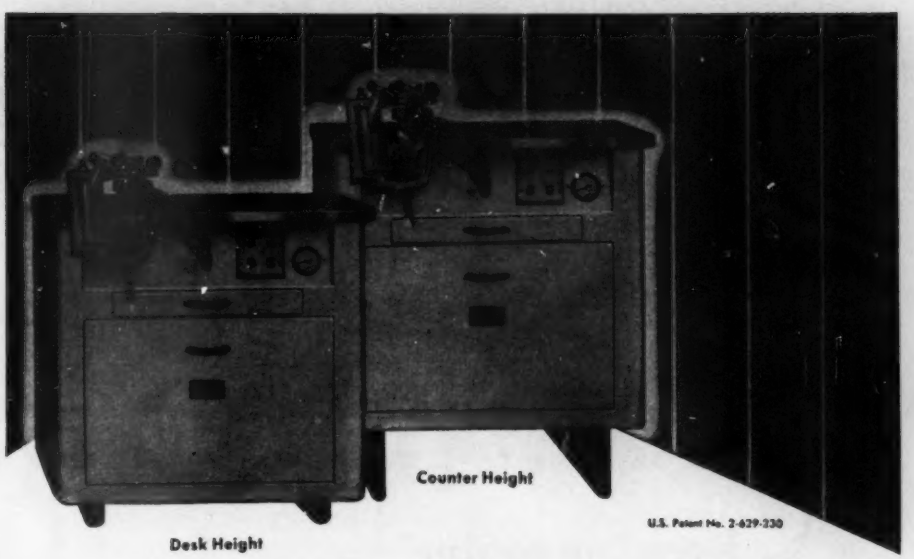
This new textbook of embryology intends to give a survey of the modern, biologically oriented embryology. The author states in the preface that his purpose is to combine comparative and experimental embryology in a new viewpoint of ontogenesis. Much emphasis is given to results of embryological-physiological research. The book is divided into two parts, a general part covering conditions of embryonal development, and the second part dealing with organogenesis. Much of the material is new and includes recent concepts in this field. The numerous illustrations are well prepared and informative. Of special interest are the complete references at the end. The book is highly recommended for those interested in fundamental concepts of embryology.

Histologische Geschwulstdiagnostik. By A.v. Albertini, Professor of Pathology of the University of Zurich, Switzerland. Price, $23.40. Pp. 544, with 638 illustrations. Georg Thieme Verlag, Herdweg 63, Stuttgart, Germany, 1955.

This book is the first edition of a "systematic morphology of the human tumors as basis for clinical judgment," and is meant to serve as an introduction and classification of oncology in general. The introductory chapter deals with histogenesis, morphology, and terminology, followed by histological diagnosis of tumors of particular organs. A classification of neoplasms of the central nervous system is not included. Clinical correlations are but minimally considered. The black-white illustrations are well selected and of excellent quality. The references to the literature are mostly to old publications, indicating the essentially descriptive nature of the book.

Textbook of Endocrinology. Edited by Robert H. Williams, M.D. Second edition. Price, $13.00. Pp. 776, with 175 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1955.

This second edition of the "Textbook of Endocrinology," which first appeared in 1950, has been brought up-to-date and extended in some respects. The chapter on obesity, originally written by Newburgh, has been revised by Daughaday. Forsham and Thorn have rewritten the sections on hyperadrenocorticism and hypoadrenocorticism, originally written by Kepler. As the editor says, the object in revising the book was to present material as concisely and authoritatively as possible. In doing so, attempts were made to minimize controversial issues. He has also tried to include as many facts and ideas as possible from fundamental studies in physiology, biochemistry, and other basic sciences. In fact, Chapter 1 deals mainly with general principles of endocrinology. In the succeeding chapters there is discussion of the pituitary, thyroid, adrenals, testes, ovaries, pancreas, and the parathyroid glands. It is of interest that no discussion is given for the diseases of the thymus or of the pineal gland. There are also chapters on the influence of the endocrine glands upon growth and development, the problem of obesity, and the subject of neuroendocrinology. Finally, there is a chapter dealing with laboratory diagnosis and assay features, and one on the diagnosis and treatment of endocrinopathies and hormone preparations. The book is well printed, and the index is excellent. In addition, there are numerous references to recent literature in relation to many of the subjects discussed. The book contains one hundred seventy-five figures, mostly photographs, microphotographs, and charts. Most of the authors are well-known contributors to the subject of endocrinology. In this book medical students, residents, and physicians will find much of value, which is presented competently.



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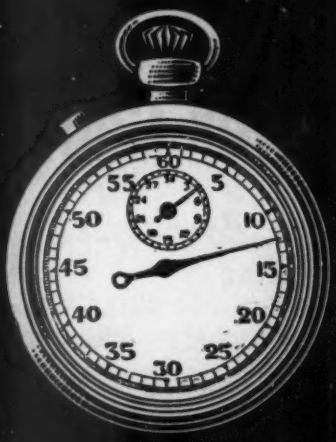

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References: 1. Hodes, M. E.: Clin. Chem. *5*:59 (Aug.) 1953. 2. Wollenweber, H. L.: Current M. Digest *21*:109 (March) 1954. 3. Oktavec, W. A., Jr., and Smetana, E. J.: Tech. Bull. Registry M. Tech. *24*:28 (Jan.) 1954. 4. Oktavec, W. A., Jr., and Smetana, E. J.: Am. J. Clin. Path. *24*:250 (Feb.) 1954. 5. Dubos, R. J.: Bacterial and Mycotic Infections of Man, ed. 2, Philadelphia, J. B. Lippincott Company, 1952, p. 371.

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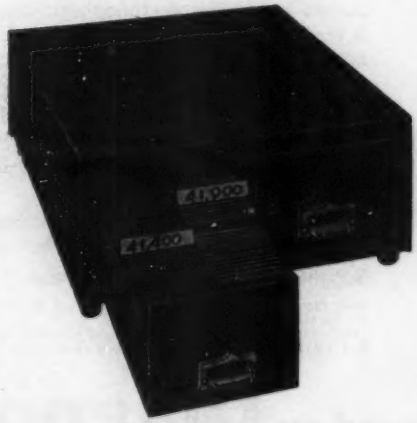
All Paragon Tray Drawer Cabinets are manufactured in standard sizes so that any number of sections may be interlocked to form one cabinet to accommodate any number of varied slides. The dimensions of the different cabinets are the same as to length and width, varying only in height. The cabinet formed by interlocking may be 18¾ x 15¾; 18¾ x 11 or 18¾ x 5 or it may be a pyramid with the sections varying in width.



C221—Capacity 1500 Slides—18¾ x 11 x 3¾
For Filing KODACHROME TRANSPARENCIES and 2 x 2" SLIDES

SPECIFICATIONS: All Paragon Tray Drawer Cabinets are made of reinforced steel construction, olive green finish. Interlocking device enables several units to be joined into one. Each sectional unit contains removable drawers with hand grip in front and rear. Interlocking steel base obtainable whenever required. **Constructed according to rigid specifications—not merely adapted.**

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